POROUS VITALIUM-BASE NANO-COMPOSITE FOR BONE REPLACEMENT

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***Abstract*—Porous nano-composites were successfully prepared on addition of 58S bioactive glass to Co-base alloy with porosities of 37.2–58.8% by the combination of milling, space-holder and powder metallurgy techniques. The results of X-ray diffraction analysis showed that induced strain during milling of the Co-base alloy powder and also isothermal heat treatment during sintering process led to HCP2FCC phase transformation which affected mechanical properties of the samples during compression test. Field emission scanning electron microscopy images showed that despite the remaining 58S powder in nanometer size in the composite, there were micro-particles due to sintering at high temperature which led to two different apatite morphologies after immersion in simulated body fluid. Calculated elastic modulus and 0.2% proof strength from stress–strain curves of compression tests were in the range of 2.2–8.3 GPa and 34–198 MPa, respectively. In particular, the mechanical properties of sample with 37.2% were found to be similar to those of human cortical bone. Apatite formation which was identified by scanning electron microscopy (SEM), pH meter and Fourier-transform infrared spectroscopy (FTIR) analysis showed that it could successfully convert bio inert Co-base alloy to bioactive type by adding 58S bioglass nano-particles. SEM images of cell cultured on the porous nano-composite with 37.2% porosity showed that cells properly grew on the surface and inside the micro and macro-pores**

***Index Terms*—Porous, Nano-composite, Milling, Mechanical properties, Bioactivity, Cell culture**

I. INTRODUCTION

The Co-base alloys conforming to the ASTM F75 (vitalium) have been widely used for biomaterials because of their appropriate biocompatibility and high mechanical properties (Ping et al., 2006; Dourandish et al., 2008; Giacchi et al., 2011). But, themismatch between elastic modulus of this alloy (240 GPa) and human bone (cancellous: o3 GPa and cortical bone: 3– 20 GPa) is a major problem which lead to stress-shielding and micro-movement between the implant and the adjacent bone and finally promotes separation of implant from surrounding tissue after implantation (Jurczyka et al., 2011; Torres et al., 2012).

In the recent years, particular attention was paid to the synthesis of porous metals, with a unique combination of metals and foam properties, to allow the ingrowth of bone tissue, which improves the mechanical fixation of the implant at the implantation site and also provide a system that enables stresses to be transformed from the implant to the adjacent tissue (Schneider et al., 1989). In addition, because porous metals have lower elastic modulus rather than bulk metals (Gibson and Ashby, 1999), using these materials is a way to overcome stress-shielding and micro-movement between the implant and surrounding bone.

Ball milling can be considered as a solid state powder metallurgy process in which an important amount of defects such as dislocations,vacancies, stacking faults are created during milling. These defects and diffusion at the atomic level which activated by high-energy ball milling, allows production of various non-equilibrium phases.

Generally, the methods for porous metal fabrication are categorized into four groups of production: liquid, solid, gas and aqueous solutions, which among of them powder metallurgy techniques, are promising because of their considerable advantages. The space-holder technique which classified as a powder metallurgy process was first employed by Zhao and Sun (2001) for producing porous aluminum. In this method, the spacer particles create the space within the structure and therefore

allow simple and accurate control of pore fraction, shape and interconnectivity in the structure.

Considering benefits of bio-glass nano-particles and porous materials, the aim of this work was to fabricate porous Co-base nano-composite using milling, space-holder and powder metallurgy techniques. The present study focused on the structural, mechanical and in vitro biological characteristics of fabricated samples.

II. MATERIALS & METHODS

2.1. Raw materials and sample preparation

Co-Cr-Mo (Co–28%Cr–5%Mo; all in weight percent) powder according to ISO standard 58342-4 (E) (ISO-1996) was pur-chased from Carpenter Co., Sweden. As shown in Fig. 1, this powder has spherical morphology with a mean diameter of about 130 mm. This powder was milled in a PM 4000 Retch planetary ball mill, using a rotational speed of 300 rpm. The 150 mL stainless steel container was charged with 12.7 g of the powder and three different stainless steel balls in size; the whole number of balls were eight: four balls with 10 mm, two balls with 19 mm, and two balls with 21.3 mm indiameter. The milling experiments were done with a ball to powder weight ratio of 12:1 with 3 cc ethanol as process control agent (PCA) under high purity argon gas. The powder after 12 h of milling was employed tofabricate the porous Co-base nano-composite

Figure 1: SEM Micrograph of .Gas Atomized Co-base Alloy Powder

The 58 S bio-glass nano-powder (58%SiO2–38%CaO–4%P2O5; all in mol%) was synthesized by sol–gel method (Taghian Dehaghani et al., 2015) and used as a bioactive material. Transmission electron microscopy (TEM, CM120; Philips, Eindhoven, the Netherlands) of the synthesized 58S bio-glass nano-powder is shown in Fig. 2. As can be observed, the sizes of these particles are approximately smaller than 100 nm and show quasi-spherical morphology.

Ammonium hydrogen carbonate (NH4HCO3) having nearly cubic shapes, distributed in the range of 250–500 mm (Fig. 3), and PVA solution (5 wt% PVAþ95 wt% water) were used as space holder and organic binder materials, respectively.

The ball milled powder, 15 wt% of bio-glass nano-powder, different amount of NH4HCO3 and two weight percent of PVA solution were mixed properly. The blended powders were then cold compacted at pressure of 200 MPa into cylindrical green compacts with 5 mm in diameter and 7.5 mm in height. The green compacts were sintered under high purity argon gas through two steps. The samples were heated at 175 1C for 2 h and 1250 1C for 2 h to burn out the space-holder particles and sintering, respectively.

2.2. X-ray diffraction analysis

The structure and phase composition of as-received, milled powders and sintered samples were characterized by means of Philips X'pert-MPD X-ray diffraction instrument with Cu Kα radiation (λCuKα ¼0.154186 nm at 30 mA and 40 kV) in the range of 301o2θo1001 (step size: 0.05 and time per step: 1 s).

Figure 2: Transmission electron microscopy (TEM) image of 58S bio-glass nano-particles

Figure 3: SEM micrograph of ammonium hydrogen carbonate powder

2.3. Physical and mechanical measurements

The total porosity of the porous nano-composites was calculated by the following formula:

$$P= (1- \rho/\rho_{th}) \times 100$$

Where, P is the total porosity, ρ is the apparent density of the porous samples and ρ_{th} is the theoretical density which is equal 6.2 g/cm3 (based on the mass percentage of respective composition).

Compression tests of samples were carried out at room temperature using a universal testing machine (UTM)(Universal HOUNFIELD, H25Ks, England) according to ASTM: E9-09. More than three samples with constant relative density were tested at a cross speed of 0.5 mm/min to measure average elastic modulus of porous nano-composite as a function of relative density. The slope of the initial linear portion 0.2% proof strength of the stress–strain curves were calculated to be as elastic modulus and yield poit of each sample.

2.4.Microscopic characterization

Scanning electron microscope (SEM, Philips XL 30) and optical microscope (Nikon, Epiphot, Japan) were utilized in order to investigate size, morphology and distribution of pores. In addition, Field emission scanning electron microscope (FESEM, Hitachi S4160) with energy dispersive spectrometer (EDS) was utilized to investigate the effect of sintering process on the size and distribution of 58 S bio-glass nanopowder.

2.5. Bioactivity evaluation

To evaluate the bioactivity of nano-composite with 37.2% porosity, Kokubo solution was used as a simulated body fluid (SBF). This solution was prepared according to Kokubo's protocol (Rehman et al., 1994), by dissolving appropriate of the relevant reagent grade chemicals in deionized water. Each sample was immersed in SBF solution in one sterilized polyethylene bottle. The immersion test was carried out in a shaking water bath under physiological condition of pH 7.41 at 36.570.5 1C for 3, 7, 14, 21 and 28 days. After SBF soaking, the samples were filtered, gently rinsed with distilled water and dried at room temperature before characterization.

The changes in pH value of the solution after preselected immersion times were evaluated by a pH meter. SEM and Fourier transform infrared spectroscopy (FTIR-Tensor 27, BRUKER) were utilized to confirm the apatite formation on the surface of samples. The absorbance spectra were collected in the frequency range of 600–2000 cm 1 with 1 cm 1 resolution.

2.6. In vitro cell culture

In vitro biocompatibility behavior of porous composite with 37.2% porosity was evaluated for incubation period of 3 and 5 days using Fibroblast L929. For this purpose, 2 104 cell/well were seeded onto the a glass cover slip as a control and sterilized sample in RPMI 1640 (Invitrogen, Eggenstein, Germany) growth media supplemented with 10% FCS, 100 units/mL penicillin and 100 mg/mL streptomycin at 37 1C under an atmosphere of 5% CO2 and 95% air incubator.

The MTT assay using 3(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT, SIGMA, USA) to measure cell viability and proliferation was utilized to quantitatively evaluate the number of viable cells that had attached and grown on the samples. The MTT solution was added to each sample and the cells were incubated for 4 h at 37 1C and the medium was replaced with dimethylsulfoxide (DMSO) and kept for 10 min. The absorbance of each sample was read at a wavelength of 545 nm by a plate reader. Statistical analysis was conducted to evaluate the difference in cell viability bythe analysis of variance (ANOVA) and po 0.05 was considered statistically significant.

Cell morphology was observed after 3 and 5 days of incubation by SEM. For SEM observation, cultured samples were rinsed with 0.1 M phosphate-buffered saline (PBS) and fixed with 2.5% glutaraldehyde. Fixed samples were then dehydrated in an ethanol series (30%, 70%, 90%, 96%, and

100%) for 10 min followed by a hexamethyldisilane (HMDS) drying procedure.

III. RESULT AND DISCUSSION

Fig. 4 indicates the XRD patterns of metal powders before milling and after 12 h of milling. As it can be seen before milling, the pattern of the metal powder shows sharp strong peaks related to the FCC phase. After 12 h of milling, the pattern of the powder shows peaks corresponding to the HCP phase. According to Eq. (1), the integrated intensities of the ð1011Þ and (200) XRD peaks of the HCP and FCC phases were used to calculate the amount of transformed HCP phase, which are 0 and 100 wt% for as-received and after 12 h of milling, respectively.

Fig. 5 shows the morphology of powder after 12 h of milling. This figure shows that powder particles intensively become flattened and particle sizes decrease.

Beside the decrease in particle size of the powders and induced defects during milling that improve atomic diffusion, resulting in faster interparticle neck growth during sintering process, FCC-HCP phase transformation is not appropriate and reduces the mechanical properties of final samples. HCP metals, having few active slip systems, are normally quite brittle (Calister, 2001).

Figure 4: X-ray diffraction patterns of Co-base alloy powder after (a) 0 h, and (b) 12 h milling times.

Figure 5: SEM image of gas-atomized Co–Cr–Mo pre-alloyed powder after 12 h milling time.

Porous nano-composite samples were successfully fabricated with different amount of total porosity, which includes both closed and open porosity, depending on the amount of NH4HCO3 as space-holder material (Fig. 6).

Fig. 7 indicates the optical images of the polished cross-section samples with different porosities. Pore morphology and statistical analysis of pore size distribution of the produced sample with 37.2% porosity are shown in Fig. 8. As can be seen from optical images, most of the large pores or in another word macrospores in sample containing 37.2% seem to be isolated from each other, but with increasing the porosity percent (Fig. 7b–d) the interconnectivity of the samples increases so that the porous sample with porosity 58.8% has nearly full interconnectivity.

Figure 6: Photograph of samples with different porosities.

Both micro-pores and pore interconnectivity are very critical factors for porous material, as they provide pathway for body fluid, nutrient transportation and also allow cells to grow inside the material (Shirtliff and Hench, 2003; Oh et al., 2006; Jones et al., 2006a,b). However, the porosity leads to change in the mechanical properties of samples which will be discussed in the next section.

The compressive stress–strain curves of samples with porosities of 37.2–58.8% are illustrated in Figs. 9 and 10. These curves show the typical compression stress–strain behavior of porous materials which consists of three stages of deformation: stage one is linear elastic region where the cell wallsdeform elastically, followed by a yield point. Stage two is the plateau region with nearly constant flow stress even with increase in stress value. Finally, in stage three or densified region the stress increase sharply in which porous collapsing is observed to occur and the stress–strain compression behavior of the sample transforms into that of bulk materials. However, the shape of strain–stress curves and also the deformation mechanism of samples are almost identical to those of other porous materials as reported in literature (Bansiddhi and Dunand, 2008; Aydogmus and Bor, 2009; Mondal et al., 2009; Mansourighasri et al., 2012; Mondal et al., 2013), but as can be seen, the stage two is not clearlyvisible especially for samples containing 37.2% and 46.6% porosity.

Figure 7: Optical micrographs of samples with different porosities: (a) 37.2%; (b) 46.6%; (c) 52.9%; (d) 58.8%.

This mechanical behavior can be related to the microstructural observations. As aforementioned, at lower porosity percent of 46.6% there is a transition from the open pores to closed pores morphology, leading to the increase in the thickness of pore walls which could carry more stress upon loading. On the other hand, the shape and smooth character of all curves show the excellent ductility of samples during compression test which can be related to the excellent formation of interparticle bonding and also to thetransformed phase during at high temperature. Fig. 11 indi-cates the X-ray diffraction of porous sample at 1250 1C for 2 h. As can be seen, this pattern not only indicates that there are sharp peaks related to the FCC phase, but also in compression with Fig. 4b shows that complete HCP-FCC phase transfor-mation has occurred. In addition to strain-induced HCP-FCC phase transformation athermal and isothermal heat treat-ment could lead to this phase transformation in Co-base alloys (Remy and Pineau 1976; Huang and Lopez, 1999a,b; Saldivar-Garcia et al., 1999; Lopez and Saldivar-Garcia, 2008).

Figure 8: (a) SEM micrograph of sample with 37.2% porosity; higher magnification micrograph showing (b) a macro-pore and micro-pore surrounded by walls and (c) statistical analysis of pore size distribution in (a).

Figure 9: Stress–strain curves of porous samples with 37.2% and 46.6% porosities

Fig. 10 – Stress–strain curves of porous samples with 52.9% and 58.8% porosities

Transformation of FCC2HCP phase during isothermal heat treatment depends on time, temperature and chemical com-position of Co–Cr–Mo–C alloy (Taylor and Waterhouse, 1983; Montero-Ocampo et al., 1999; Saldivar-Garcia and Lopez, 2001; Ramıırez-Vidaurri et al., 2009). Pure cobalt shows an allotropic transformation (FCC2HCP) at 417 1C which some element such as Cr increases this transformation tempera-ture (TC). For instance, TC is shifted toward near 970 1C in Co– 27Cr–5Mo–0.05C wrought alloys (Adams and Altstetter, 1968; Saldivar-Garcia et al., 1999). In this investigation, owning to higher sintering temperature above TC, full isothermal HCP to FCC phase transformation has occurred.

The elastic modulus and yield strength which were calculated from the slope of elastic deformation region and 0.2% proof stress are listed in Table1. This table as expected shows that the elastic modulus and yield strength decrease with increasing porosity. The results of the elastic modulus and 0.2% proof strength values indicate significant improvement taking place in the mechanical properties of Co-base alloy. These values, especially elastic modulus values, are significantly lower than those of the cast Co-base alloy with elastic modulus of about 240 GPa. Metallic implants, having elastic modulus higher than of natural bone, carry the induced stresses at the tissue-implant interface and this can lead to impairment of the adjacent tissue (bone) and loosen the prosthesis. Oksiuta et al. (2009) have demonstrated that Co-base composites containing 5–15 wt% of the bio-glass have lower elastic modulus values of 143.8–125.2 GPa. In another work, Bahrami et al. (2015) have shown that with an increase in hydroxy apatite (HA), a decrease in the Young's modulus of the Co-based composites was observed. They have reportedthat Co–Cr–Mo composites containing 10, 15 and 20 wt% of HA have the elastic modulus values of 112, 100 and 89 GPa, respectively. It is worth to mention that, obtained values of elastics, in this research, are significantly higher than those of the natural bone. However, here, the mechanical proper-ties of sample with 37.2% porosity, having the elastic mod-ulus and yield strength of 8.3 GPa and 198 MPa, respectively, are almost the same as that of natural bone.

Figure 11: X-ray diffraction pattern of porous sample sintered at 1250 1C for 2 h.

Figure 12: Relative Young's modulus as a function of relative density

According to the obtained equation of fit curve in Fig. 12 on a logarithmic scale, the relationship between the relative elastic modulus (Ep/ES) on the relative density (ρp/ρs) where the subscript 's' represents the bulk properties and the sub-script 'p' represents the measured porous sample, is expressed to be as following equation:

$$EP/ES = 0.15(\rho P/\rho S)^{2:48} \quad (3)$$

Also in Fig. 12, the relative elastic as a function of relative density is compared with predictions of Gipson and Ashby (G&A) model (1999). In the G&A model which has been applied to polymer, ceramic and metals, the relationship between the elastic modulus and normalized density is as following equation:

$$EP/ES = A\,(\rho P/\rho S)^{n} \quad (4)$$

In this equation n is density exponent and A is geometric constant which are equal to 2 and 1 respectively, for rigid elastomers, polymers, glasses and metals.

As can be seen in Fig. 12, both lines of G&A model and experimental values show the similar trend, but experimental values are lower than the elastic predictions by G&A model. Comparison between Eq. (3) and Eq. (4) shows that values of A and n constants are significantly different from each other. Values of A and n vary over a wide range in different literature (Yamada et al., 2002; Wang et al., 2009). These differences could be related to the some simplified assumption like uniform pore size and struts, smooth pore walls and pore edge, no defects in pore walls no change in microstructure and so on, that were taken in development of this model. Indeed in the G&A model the structure of porous sample are assumed to be ideal. However the optical and SEM images samples in this study shown considerableinhomogeneity of the structure and there are highly non-uniform struts that they lead to decrease in elastic modulus of porous sample.

The immersion test study, which is a measure of the bioactivity of the material (Kokubo and Takadama, 2006) was successful in confirming the ability for apatite formation on the surface of the nano-composite with 37.2% porosity. Fig. 13 shows SEM micrographs of the sample after 28 days of immersion in SBF. It is obvious that the bone-like apatites on the surface, having two different morphologies, are formed as the result of contacting the porous nano-composite with SBF. These figures demonstrate that both bright needle-like (hexagonal shape) and nearly cubic-like phase which are in good agreement with the results of other researchers (Kokubo and Takadama, 2006; Bahrami et al., 2015; Paital and Dahotre, 2009), are precipitated from the bioglass after immersion test. Fig. 14 shows the distribution of 58S bio-glass nano-particles in the nano-composite following by EDS spectra of these particles. Fig. 15 show SEM image and EDS maps of porous nano-composite surface indicating 58S bio-glass micro-particles. These images demonstrate thatdespite the 58S bio-glass nano-particles, there are also some 58S bioglass micro-particles which have been formed due to high surface area of nano-particles during sintering process. Indeed, nano-particles with high surface area to volume ration tend

to agglomerate especially in high temperature. On the other hand, it has been reported that the crystallization of 58S bio-glass nano-powder start to occur at temperature of 918 1C and crystallized of 58S bio-glasss nano-powder shows a lower bioactivity than of amorphous nano-powder (Taghian Dehaghani et al., 2015). However, the needle-like apatite which is similar to apatite in bone struc-ture (Paital and Dahotre, 2009; Pattanayak et al; 2011) and also nearly cubic-shape apatite could be related to the nucleation and growth of apatite in SBF from crystallized nano-powder and from agglomerated 58S bioglass, respectively.

Figure 13: SEM micrographs of porous nano-composite after 28 days immersion in SBF solution, showing (a) and (b) different hydroxyapatite morphologies.

Figure 14: (a) Distribution of nano-58S bio-glass in the nano-composite observed by FE-SEM and (b) EDS spectra of these particles.

Confirmation of apatite formation was obtained by the pH values measurement of SBF solution and FTIR analysis. pH trends for the nano-composite with 37.2% porosity and with-out any soaked sample in SBF as a function of immersion time are shown in Fig. 16. pH values for SBF without sample is approximately stable during immersion times. The trend ofpH values of nano-composite is similar to that reported for 58 S bioglass powder (Taghian Dehaghani et al., 2015) and so can be explained by three stages: the stage one of the diagram is the increasing region which ends after 7 days of immersion time. Stage two indicates the gradual decrease of pH, but the pH value does not reach the initial value. In stage 3, the change of pH is negligible in longer days (14–28 days). pH increase in stage one is due to ion exchange between Caþ2, Pþ5 and Siþ4 in the sample and protons (Hþ or H3Oþ) in the solution. Migration of Caþ2 and PO 3 ions to the surface and also consuming OH 1 ions in the formation of HCA phase (Shirtliff and Hench, 2003; Jones et al., 2007) lead to decrease of pH in stage 2. Finally, in stage 3, equilibrium between dissolution and precipitation and therefore equal ion exchange from solution and sample results in stable of pH values.

Figure 15:SEM image and EDS maps of porous nanocomposite: (a) SEM image, (b) Si map, (c) Co map, and (d) Ca map.

Figure 16: pH reacted SBF solution for porous nano-composite with 37.2% porosity versus days of immersion.

For the aforementioned sample the functional groups of the bright particles nucleated on the surface were investigated using FTIR. FTIR spectra in the range 600–2000 Cm 1 was taken from the surface of the sample to ensure detection of any apatite formation (Fig. 17). This spectrum approves that the precipitates in the form of bright particles, occur as carbonated hydroxyl apatite (HA) with its characteristic P–O peaks along with as C–O bands. Well-defined phosphate peaks could be seen at 1050 and 605 Cm 1 indicating the phosphate absorption in crystalline state and especially the bands at around 605 Cm-1 are characteristic feature of PO4 3 in the apatite lattice (Li et al., 1992). The carbonate peaks observed at 1553 and 905 Cm 1 indicate that HA contained some CO3 2 groups in PO4 3 sites of apatite lattice (Koutsopoulos, 2002). In addition, two peaks at 657 Cm 1 and 1626 Cm 1 are also observed in the spectra which have been assigned to silicate groups of bio-glass and absorbed water, respectively.

The MTT assay was used to determine fibroblast cell proliferation on the samples with 37.2% porosity. Fig. 18 shows a comparison of cell densities on porous samples and control after 3 and 5 days of culture. The numbers of cells increase with increasing incubation time. The statistical analysis which was carried out by analysis of variance(ANOVA) and po0.05 was considered statistically significant, shows that cell numbers on the samples were significantly higher than those on the controls at 3 and 5 days. This is due to porous structure and therefore cells can attach and proliferate on the surface and inside the material. The above results also confirm the bioactive potential of the scaffolds as a substrate for cell binding (Mishra et al., 2009). Previous investigations have shown that bioactive glass stimulates the secretion of angiogenic growth factors in fibroblasts (Day et al., 2004; Day, 2005; Keshaw et al., 2009) and the prolifera-tion of endothelial cells (Keshaw et al., 2005; Leach et al., 2006).

Figure 17: FTIR spectra of porous nano-composite after 28 days of immersion in SBF solution.

Figure 18: MTT assay of L929 fibroblasts cells observed on Control and sample surfaces as a function of culture time.

SEM images of cell cultured on the porous nano-composite with 37.2% porosity for 3 and 5 days are shown in Fig. 19. Fibroblasts cells have spindle-like morphology with blebs and folds in their surface membranes, but attached fibroblasts observed under SEM after 3 days of incubation are either flat or round in morphology. Arrows on Fig. 19(a) show examples of round and elongated cells and of the beginning of fila-ments formation. Round fibroblast cells are found to be poorly attached to the surface and elongated fibroblasts cells are tightly attached to

sample. Also, as shown in Fig. 19(b), cells having numerous filapedia extension, start to bridge and cover the space between particles. Furthermore, it is observed that some cells are connected to each other through vicinity cells and grow on the surface and inside the pores. As can be seen in Figs. 7, 8 and 12, there are micro and macro-pores and also nano-sized particles which are classified into micro, macro and nano-sized topologies (Jurczyka et al., 2011). These surface features which result from special structural proper-ties could affect the adhesion and proliferation behavior of cells. So that, there are reports show that porous structure or in the other word higher surface area and rough topography are beneficial for better cell migration, attachment and pro-liferation (Wennerberg et al., 1998; Postiglione et al., 2003; Saldana et al., 2006; Koutsopoulos, 2002). In addition, 58S bioglass in the nanometer range with unique surface proper-ties (such as surface energy, surface wettability and surf-ace topography) has a particular role in the adsorption of proteins, adhesion of osteoblstic cells and therefore the rate of osseointegration (Brett et al., 2004). As shown in Fig. 19(c) after 5 days of incubation, the most of the sample's surface is covered by a multilayer of flat and spread cells. In addition to the structural properties which can lead to the special surface features, 58S bioglass with particular chem-istry play an important part in the behavior of cell during cell culture. As it has been explained, the bioactivity of this bioglasss is related to the exchange ions, dissolution and precipitation. It has been reported that the release of Si which occurs during dissolution of bioglass structure in the cell culture medium can increase cell proliferation (Gough and Hench, 2004).

Figure 19: SEM micrographs of L929 fibroblasts cultured on porous nano-composite with 37.2% porosity after (a) and (b) 3 days and (c) 5 days.

IV. CONCLUSION

Porous nano-composites were successfully fabricated on addition 58S bioactive glass to Co-base alloy with porosities of 37.2– 58.8% by the combination of milling, space-holder and powder metallurgy techniques. Significant results and improvements inthe mechanical, bioactivity and biocompatibility properties of these porous nano-composites were observed which can be drawn as follow;

1.HCP2FCC phase transformation was observed to occur due to induced strain during milling of Co-base alloy powder and also at high temperature during the sintering process of samples which affected the mechanical proper-ties of the samples during compression test.

2.After sintering process, despite the remaining 58S powder in nanometer size in the composite, there were micro-particles due to sintering at high temperature which led to two different apatite morphologies after immersion in simulated body fluid (SBF).

3.The compressive stress–strain curves of the samples showed the typical ductile behavior of porous materials. Calculated mechanical properties from these curves showed that the decrease in porosity from 58.8% to 37.2% resulted in an increase in elastic modulus from 2.2 to 8.3 GPa and also an increase in 0.2% proof strength from 34 to 198 MPa. In particular, the mechanical properties of sample with 37.2% porosity were found to be similar to those of human cortical bone.

4.The elastic modulus values followed power law relation-ship with relative density, but were different from predicted by the Gipson–Ashby model.

5.Bioactivity evaluation of the fabricated porous nano-composite with 37.2% porosity showed that two different apatite morphologies were formed on the surface of the sample. It was concluded that the bioinert Co-base alloycould be successfully converted to a Co-base bioactive type by adding 15 wt% bio-glass nano-particles.

6.Results of cell cultured on the porous nano-composite with 37.2% porosity showed that cells grew on the surface and inside the micro and macro-pores. And also, it was observed that cells well connected to each other through vicinity cells.

The results of present work have indicated that porous Co-base nano-composite is a potential candidate for bone replacement under load-bearing condition due to its appropriate properties.

REFERNCES

1) Adams, R., Altstetter, C., 1968. Thermodynamics of the cobalt transformation. Trans. TMS-AIME 242, 139–146.
2) Aydogmus, T., Bor, S., 2009. Processing of porous TiNi alloys using magnesium as space holder. J. Alloys Compd. 478, 705–710.
3) Bahrami, M., Fathi, M.H., Ahmadian, M., 2015. The effect of nanobioceramic reinforcement on mechanical and biological properties of Co-base alloy/hydroxyapatite nanocomposite. Mater. Sci. Eng. C 48, 572–578.
4) Balagna, C., Spriano, S., Faga, M.G., 2012. Characterization of Co– Cr–Mo alloys after a thermal treatment for high wear resistance. Mater. Sci. Eng. C 32, 1868–1877.
5) Bansiddhi, A., Dunand, D.C., 2008. Shape-memory NiTi foams produced by replication of NaCl space-holders. Acta Biomater. 4, 1996–2007.
6) Brett, P.M., Harle, J., Salih, V., Mihoc, R., Olsen, I., Jones, F.H., 2004.
7) Roughness response genes in osteoblasts. Bone 35, 124–133.
8) 308 journaloftheme chanica lbehaviorofbiomedicalmater ials57(2016)297–309
9) Calister Jr., W.D., 2001. Fundamentals of Materials Science and Engineering. John Wiley & Sons Inc, New York.
10) Day, R.M., 2005. Bioactive glass stimulates the secretion of angiogenic growth factors and angiogenesis in vitro. Tissue Eng. 11, 768–777.