Sys Rev Pharm 2020;11(5):804-813
A multifaceted review journal in the field of pharmacy

Effect of Cobalt Addition on the Cytotoxicity and Cell Attachment of Titanium Alloys

Fatimah J. Al-Hasani

Department of Materials Engineering/ University of Technology\ Iraq
E mail:130141@uotechnology.edu.iq,alhassanifatimah@gmail.com

ABSTRACT

Various types of titanium base alloys are utilized as implant materials and as minis crews in orthodontics. The purpose of the current study is to enhancing titanium-based alloys with the additions of Cobalt in different percentage (5%, 10%, and 15%) to enhance primary osteoblast attachment and implant tissue incorporation. the reaction between human MG63 osteoblast-like cells and titanium – cobalt alloy face is substantial in biomaterials and tissue engineering field. The major target was to estimate the proteomic response of MG63 osteoblast-like cells in various cobalt percentages. The samples with and without cobalt addition was produced by using powder technology in order to get an acceptable initial surface roughness. Also sample characterizations includes (scanning electron microscopy (SEM), X-ray diffraction (XRD), vitro test (Biocompatibility MTT assay with MG-63 Cells). Results obtained from XRD and microstructure observations, sample without cobalt addition refer to an alloy with single α phase, while after the additions of cobalt in different percentage (5%, 10%, 15%) the samples consist of two α-Ti and intermetallic compound Ti_2Co and the amount of Ti_2Co phase slightly increase with increasing cobalt content. From MTT assays Proliferation and cells attachment of MG63 were cultured after 2 days Cells grown for the pure titanium alloys possess finer and circular confines compared with the grown cells at (titanium – cobalt) alloys with all cobalt percentages. Rising cobalt percentage makes the MG63 cell sharper and enlarges which means cobalt aid to enhancing both cell proliferation and growth. Cytotoxicity Cell Viability results was kept, exhibition no kind of attack caused by titanium or cobalt materials, The feature, of cobalt additions, in contrast to commercial pure titanium alloys, is the presence of active and non- cytotoxic compound (Ti_2Co) which presents several attractive features, make the surface more attractive to the bone .

Keywords: titanium base alloys, titanium – cobalt alloy, MG63 osteoblast-like cells, cells attachment, Cytotoxicity Cell Viability.

Correspondence:
Fatimah J. Al-Hasani
Department of Materials Engineering/ University of Technology\ Iraq
Email:130141@uotechnology.edu.iq,alhassanifatimah@gmail.com

INTRODUCTION

Metallic biomaterials are embedded in human body, so it is essential to realize their biological effects in addition to their mechanical, physical, chemical once. Titanium and its alloys meet these Requirements compared with other metallic biomaterials [1]. High biocompatibility with low dissolution in body fluids of titanium alloys aid this type of metallic biomaterials to be suitable and safe inside the body and exhibit good bio-adhesion [2]. The high elasticity (Young's modulus) of titanium alloys is the most important feature in these types of biomaterials. Young's modulus of titanium materials is around 50% of that of Co-Cr alloys and stainless steel. Specially, wide range of titanium and titanium alloys are largely utilized as mini screws in orthodontics in addition to their usage as implant materials. Consequently, numerous researches have manifested the impact of elemental additions to the pure titanium on tissues such as osteoblast-like cells [3]. Actually, the primary stages of osseointegration are influenced by the following parameters: alloy composition, and energy of titanium implant surfaces, roughness, and topography, [4]. Regardless of the long-term use of titanium-cobalt alloys as stable and removable restoration materials, there still are important questions about their behaviors inside the biological environment [5]. To discuss this possibility, prosthodontic studies should involve cell and molecular biological methods in order to estimate the host's immune, non-immune, and chronic inflammatory reactions to such alloy in contact with the oral tissues [6].There is stronger linkage between surface composition and cell number when the human osteoblast-like MG63 cells are cultured on Titanium-cobalt alloys [7]. The introduction of local factors by osteoblasts is also influenced by Alloying element percentage in titanium base alloys, for an increasing in cobalt addition to the pure titanium alloys lead to increase osteoblast production of insulin-like growth factor (IGF), bone morph genic protein (BMP)-2, transforming growth factor-beta (TGF-b) , and alkaline phosphatase, osteocalcin [8]. The reaction of immune system is followed by the introduction of several soluble materials, named cytokines. These molecules have various biological frame and they have important physiological role in bone remodeling. The major contribute of inflammatory reactions is the immunoglobulin production and Deregulated cytokine at local disease sites and they must have been considered in all cases [9]. Through the various cytokines implicated in the mobilization and regulation of body responses in inflammation, cobalt ratio in titanium base alloys surfaces appear to have a major role in the inflammatory reaction. The production of active intermetallic compound has been proving to promote various immune responses and cell viability in vitro [10]. The aim of this research is to see the compatibility of the body with the MG - 63 cells whether it was toxic or useful to create new cells, realization of the reaction between human MG63 osteoblast-like cells and titanium – cobalt alloy is substantial in the field of tissue engineering and biomaterials. The main objective was to estimate the proteomic response of MG63 osteoblast-like cells to various amount of cobalt content.

Experimental work

The experimental procedure involving the preparation of pure titanium and titanium –cobalt alloys samples with three different cobalt percentages. The samples production achieved by powder technology technique in order to creates considerable surface roughness due to the production of degree of porosity caused by manufacturing mode. Pure titanium powder 60µ in particle size and pure cobalt powder35µ in particle size with chemical composition listed in table (1),(2), were mixed in a boll mill for 15 min at medium level of speed for each sample to obtain acceptable distribution of the powders. The compaction process accomplished by hydraulic press machine, maximum applied pressure of 25 ton for five minutes and then the applied pressure was released gradually. The samples were then removed carefully from the die, the resulting compacted disc with 20 mm in diameter. Finally, the sintering of compacted samples achieved at 1100 °C for 4hours using non-oxidized atmosphere (argon gas) to prevent the oxidation of titanium and titanium cobalt samples.

Table 1. composition of Titanium powder.

Element	Percentage %	Element	Percentage %
Ni	0.019	Al	<0.003
Si	0.010	V	<0.004
Zr	0.003	Cr	0.008
W	<0.010	Mn	0.045
Ta	<0.012	Mo	<0.002
Nb	0.006	Sn	0.030
Titanium = 99.8 %			

Table 2. composition of Cobalt powder.

Element	Percentage %	Element	Percentage %
Cr	<0.0019	Ni	0.011
Mn	<0.021	Pd	<0.009
Mo	<0.009	Ru	<0.002
W	<0.01	Ta	<0.005
Cobalt = 99.8%			

The resultant was four samples in different alloy composition which listed in table (3) was characterized in several type of inspections includes; scanning electron microscopy (SEM), X-ray diffraction (XRD), and vitro test (Biocompatibility MTT assay with MG-63 Cells).

Table 3. samples composition

Sample Number	Composition
1	Pure Titanium alloy with 99% Ti
2	95%Ti – 5% Co
3	90%Ti-10% Co
4	85%Ti-15%Co

Results and Discussion

Scanning Electron Microscopy (SEM) Observation

Microstructure observation results by SEM of all samples (master sample and samples with cobalt addition was presented after ground by silicon carbide emery paper with different scales starting from 600, 800, 1000 scale to obtain smooth and scratch free surface. Finally, the samples were refined with smooth cloth and etching in a suitable solution.

Figures (1) and (2) show the surface morphologies of sample 1 (pure titanium) it evident to get a degree of porosity due to the manufacturing process.

While the microstructure of other samples reveals all samples consist mainly of two constituent (αphase + Ti_2Co intermetallic compound) structure at room temperature as shown in figures (3- 8). Comparing with the master sample, the Cobalt additions lead to increasing the bright reign (Ti_2Co) due to the effect of cobalt addition. The Ti_2Co particles appear bigger with the increasing of cobalt content as see in figures (7, 8) at 15% Co. It demonstrates that the coarse and hard Ti_2Co grains were observed at 15% Co than the smaller ones (5%Co, 10%Co) in figures (3, 4) and (5, 6). which contained at least two finely dispersed phases. This was due to a metastable or non-equilibrium metastable phase can be introduce in alpha- alloys having sufficient beta-stabilizing elements to hold the beta phase at room temperature in fast cooling from high in the α + β phase region. 100% beta can be retained by air cooling beta alloys. The retained beta (or martensite) decomposition is the fundamental of heat-treating titanium alloys to higher strengths. [11, 12]. Addition of Cobalt as alloying element leads to increasing in the light region due to the effect of Co as α stabilizer element.

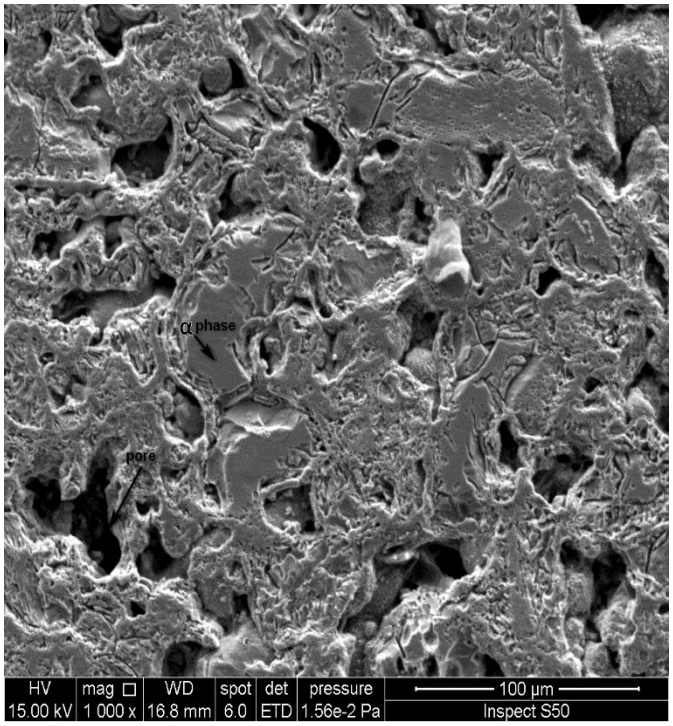

Figure 1. SEM image of sample (1).

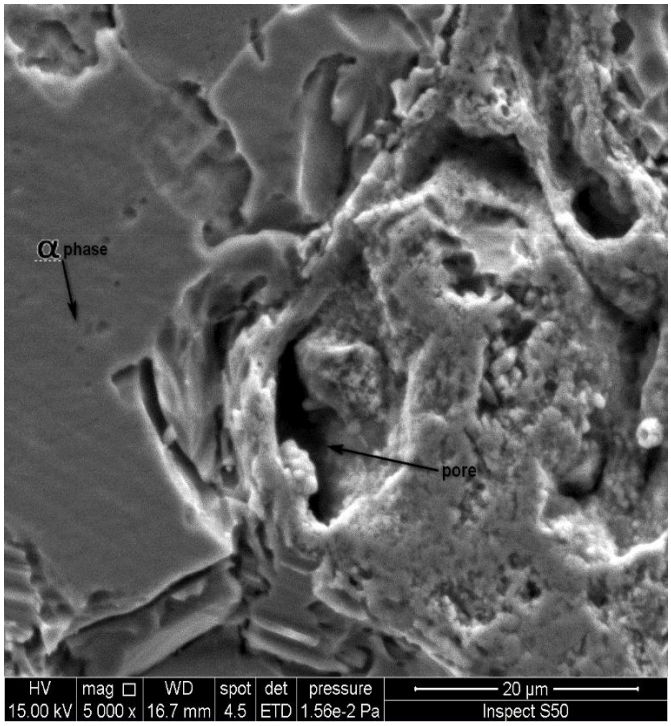

Figure 2. SEM image of sample (1).

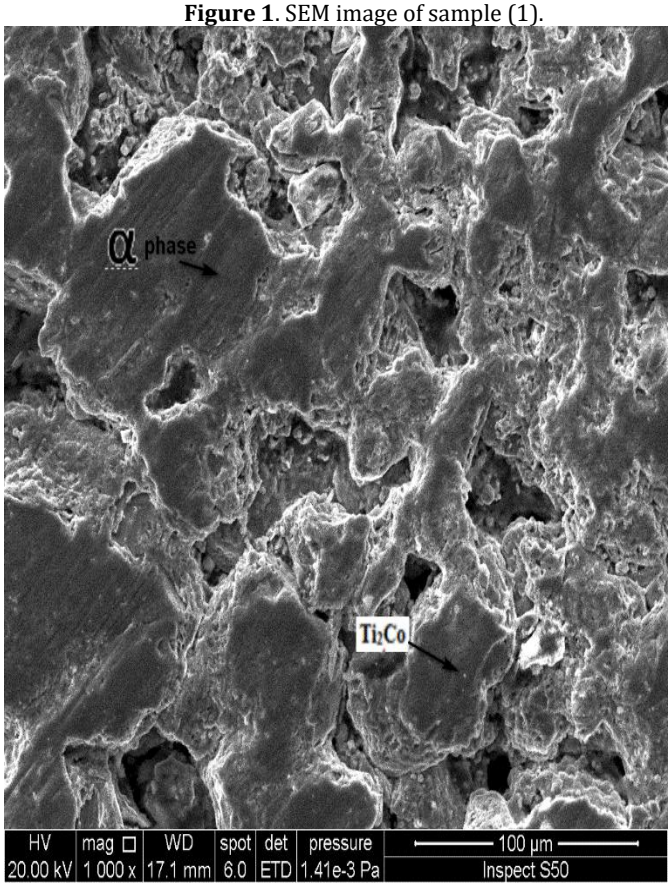

Figure 3. SEM image of sample (2).

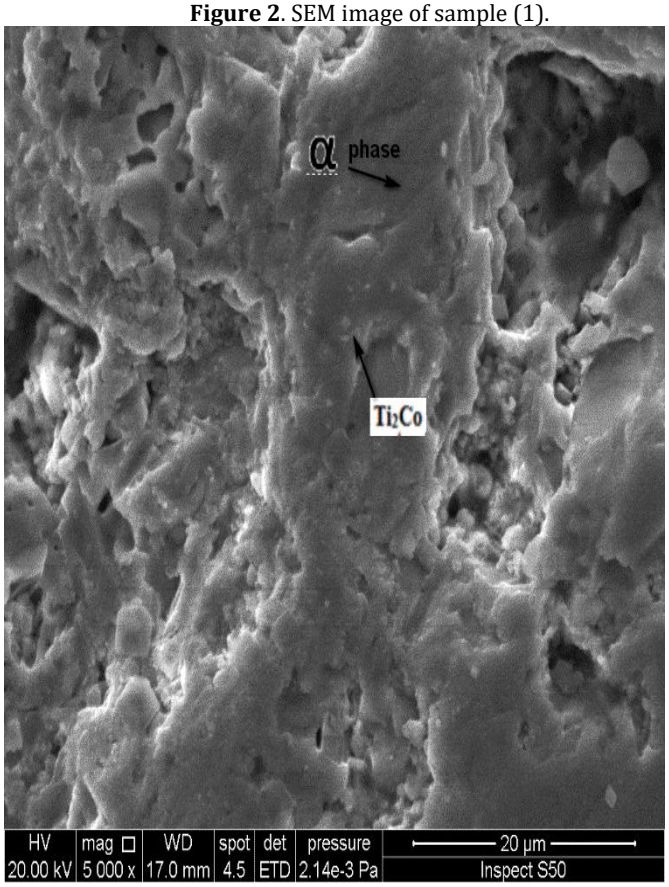

Figure 4. SEM image of sample (2).

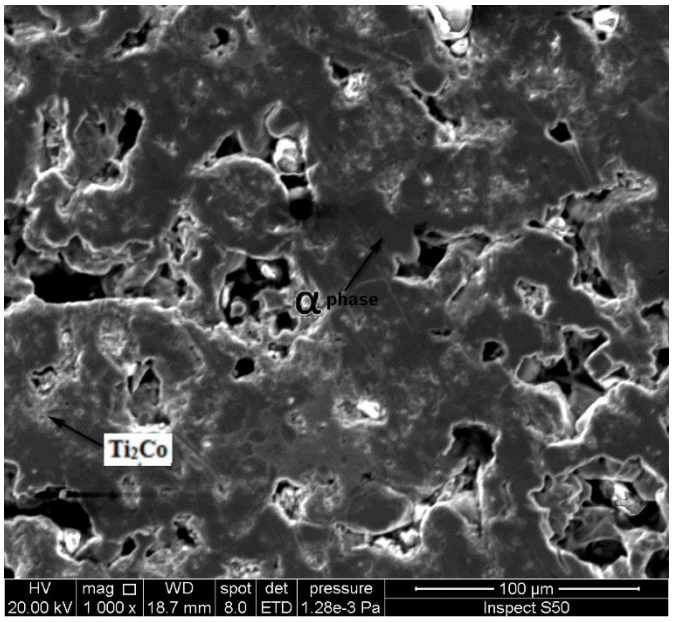

Figure 5. SEM image of sample (3).

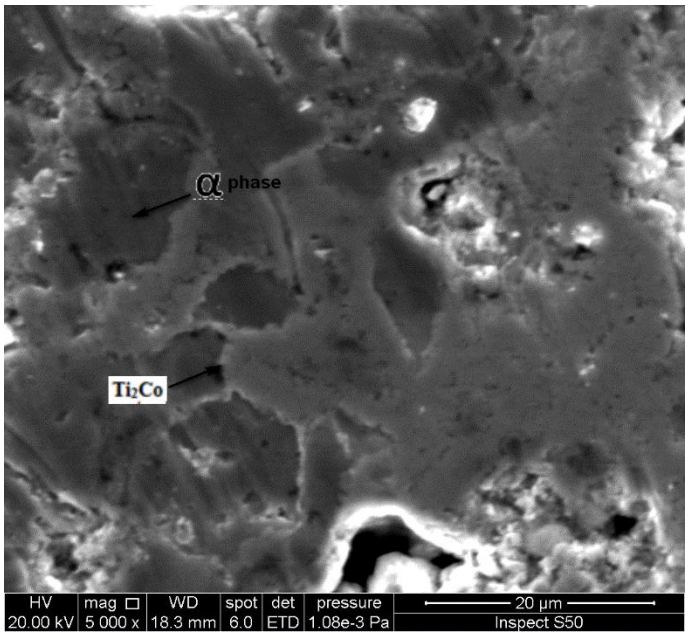

Figure 6. SEM image of sample (3).

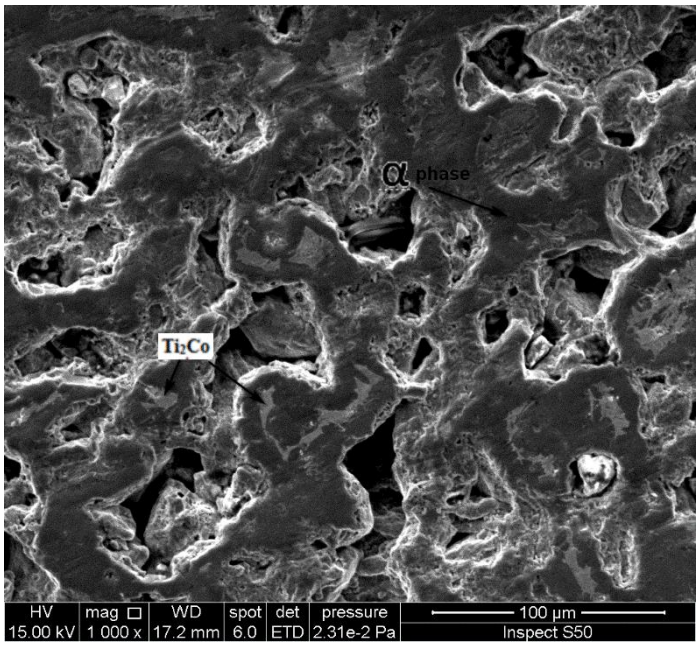

Figure 7. SEM image of sample (4).

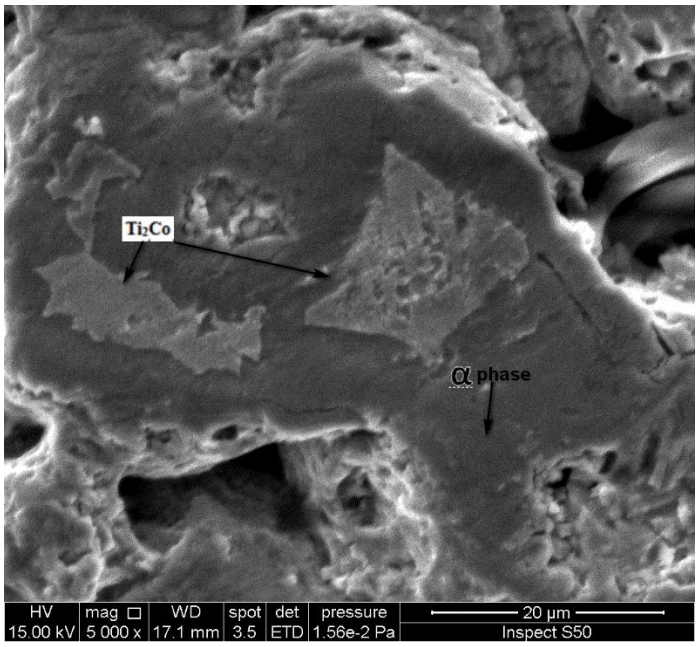

Figure 8. SEM image of sample (4).

X-Ray Diffraction

X-ray diffraction tests were done for all samples after the sintering practices. Figure (9) shows the diffraction patterns obtained for the samples were the phases that developed as a result of sintering could be detected. Figure (9) show the XRD pattern of master sample without cobalt addition which refers to an alloy with single α phase. There are likely no presents of pure metals that prove the time and temperature of sintering utilized in this work results in full sintering reactions.

While in Figures (10, 11, and12) the X-ray diffraction patterns are showing that, all samples comprise intermetallic compound Ti_2Co in addition to α-Ti phase. Because of the composition of Ti_2Co not change with the temperature. Thus, the Ti_2Co peaks positions in the X-ray diffraction patterns are same for all three samples. However, the Ti_2Co phase was slightly increased with increasing cobalt content. Due to the varying in total amount of cobalt in such alloy, and the solubility of cobalt in the α-Ti based solid solution rose when the cobalt content approached to 15%. According to the phase diagram, the cobalt solubility in α-Ti-based solid solution rising with increasing of Co content in α-Ti decreases the lattice parameter.

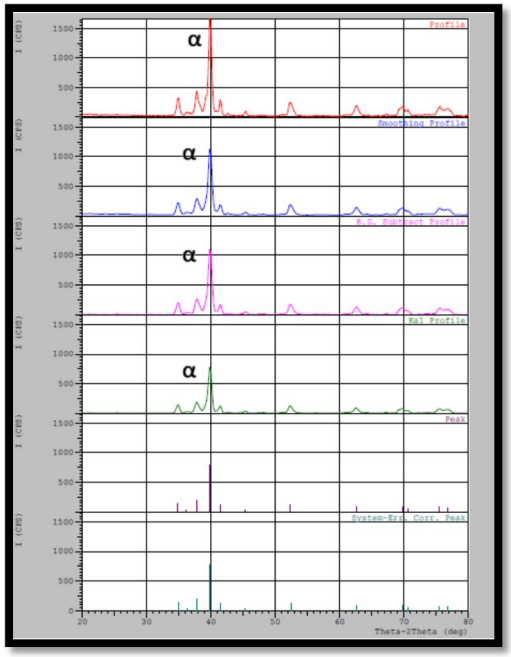

Figure 9. XRD pattern of sample (1).

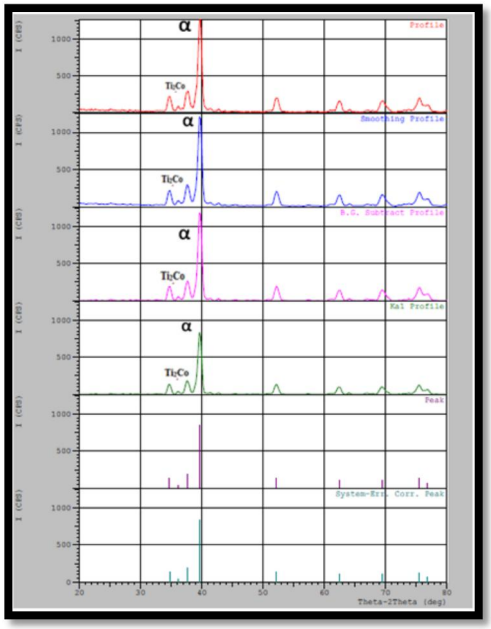

Figure 10. XRD pattern of sample (2).

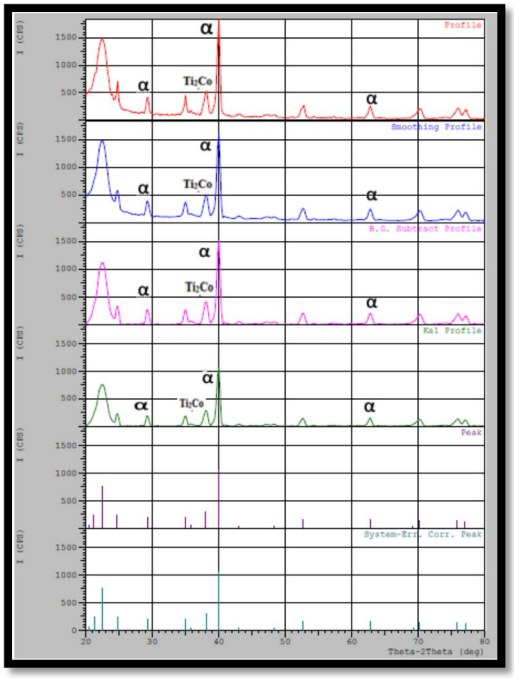

Figure 11. XRD pattern of sample (3).

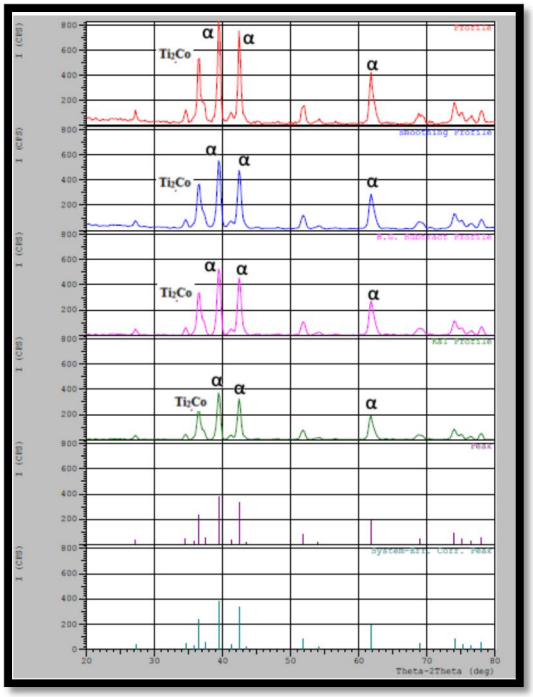

Figure 12. XRD pattern of (sample (4).

Vitro Test (MTT Assay with MG-63 Cells)

MG-63 cell lines were cultured in T-75 flask in RPMI-1640 medium containing 10% bovine blood serum (FBS and 1% penicillin streptomycin antibiotic). After cell culture in the flask and 80% of the flask floor was filled by cells, the cells were removed by the trypsin EDTA-0.25% enzyme. Cell suspension was centrifuged at 1200 rpm for 5 minutes. After centrifugation, the supernatants of the cells were discarded and the sedimented cells were suspended in the new culture medium. Cell counting was done by trypan blue staining on the neobar under the microscope. The surfaces were sterilized by immersion in 70% ethanol for 2 h. To perform cytotoxicity assay, 5000 MG-63 cells per square centimeter were cultured on the surfaces. Cells were cultured on the surfaces for 14 days to evaluate the levels of cytotoxicity and cell growth on the surface. The cultured cell sample of the plate was considered as the control sample. After 3, 7, 10 and 14 days, the supernatants of the cells were removed and MTT solution (0.5mg / ml) was added to the samples and incubated in dark medium at 37 ° C for 5.3 hours. After completion of the mentioned time, the MTT solution was removed from the plate and the resulting purple dye dissolved in DMSO. The purple absorbance at 570 nm was read by ELISA reader. MG-63 cells were cultured on the surfaces for 2 days according to the above protocol to evaluate cell adhesion. After 2 days, samples were fixed with 4% glutaraldehyde for 1 hour. After 1 hour, the glutaraldehyde solution was extracted and the samples were dehydrated with ethanol, 90%, 80%, 70%, 60% for 15 min and prepared for electron microscopy.

Proliferation and cell attachments of MG63

The first set of experiments was to determine how well cells grew (proliferated) on the surface of polished samples and the adhesion of cell on different titanium alloys. From images proliferation and cell adhesion of MG63 osteoblast-like cells were cultured after 2 days and prepared for electron microscopy. Cells grown for the pure titanium alloys as in figure (13, 14) possess a circular and finer boundary in contrast with grown cells on the (titanium – cobalt) alloys showed in figure (15, 16) and also with all cobalt percentages. Thus, the cobalt additions largely influenced in the morphology of MG63 osteoblast-like cells. An increasing in cobalt percentage make the MG63 cell more sharp and enlarge as showed in figure (17,18) thus cobalt aid to enhancing both cell proliferation and growth, due to Ti_2Co intermetallic compound formation that lead to make titanium surface to be more active . Accordingly an increasing in Ti_2Co compound rising the cellular adhesion structures, consequently may cause alterations in cellular functions this effect was highly observed in figure(19,20) in which the hole surface was covered by the rough Mg63 cells.

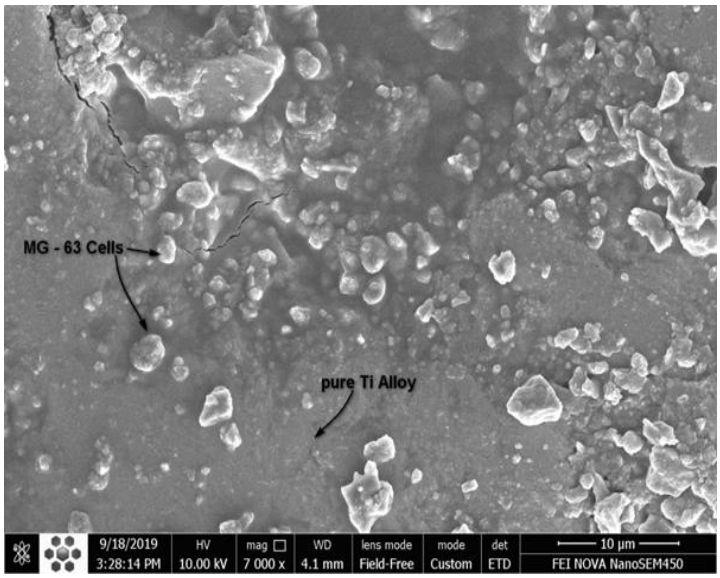

Figure 13. proliferation of MG63 osteoblast-like cells and the surface of sample (1), MG-63 cells were cultured on the surfaces for 2 days.

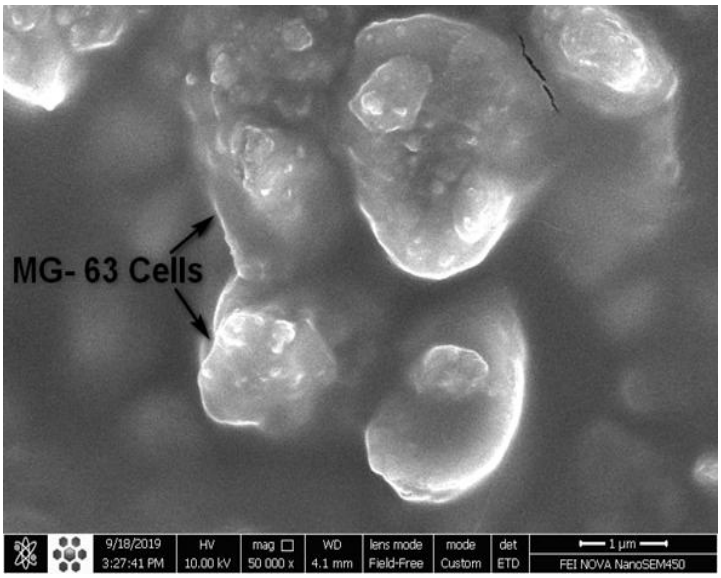

Figure 14. high magnification shows proliferation and cell adhesion of MG63 osteoblast-like cells. - MG-63 cells were cultured on the surfaces for 2 days for sample (1).

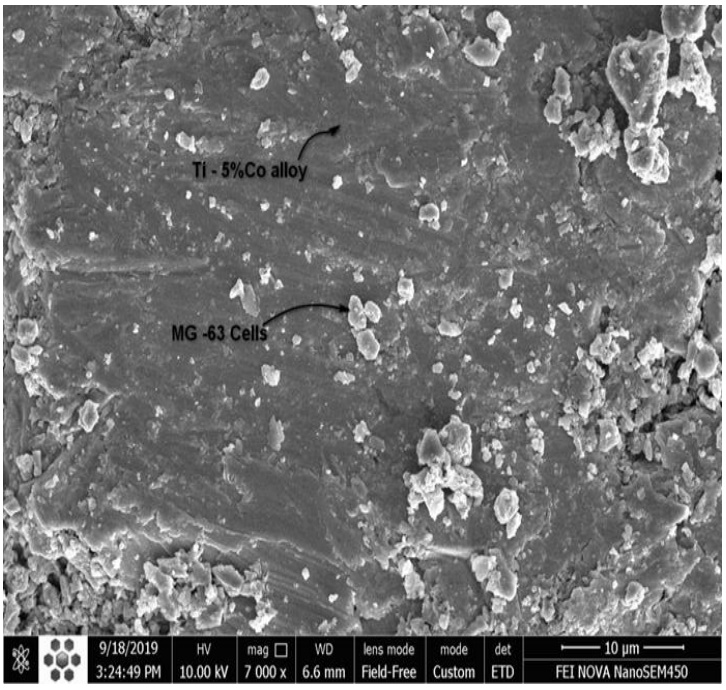

Figure 15. proliferation of MG63 osteoblast-like cells and the surface of sample (2), MG-63 cells were cultured on the surfaces for 2 days .

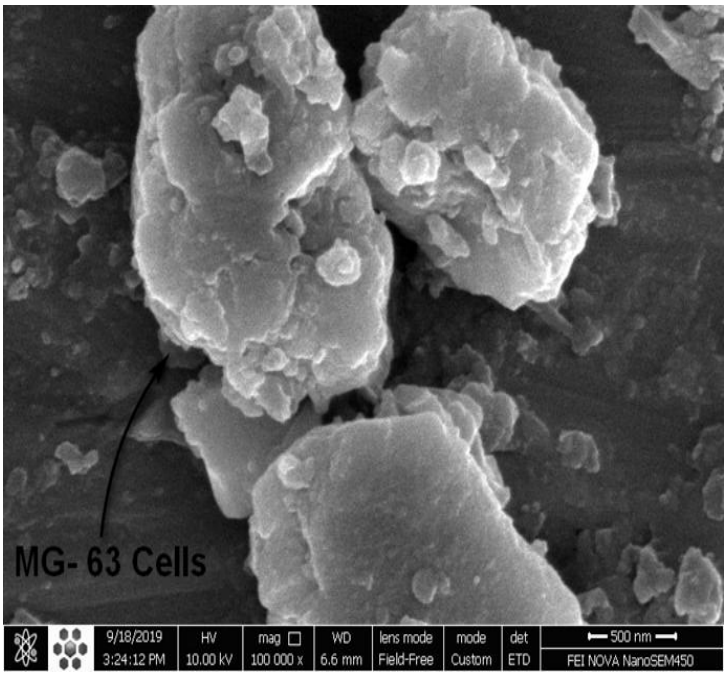

Figure 16. high magnification shows proliferation and cell adhesion of MG63 osteoblast-like cells. - MG-63 cells were cultured on the surfaces for 2 days for sample (2).

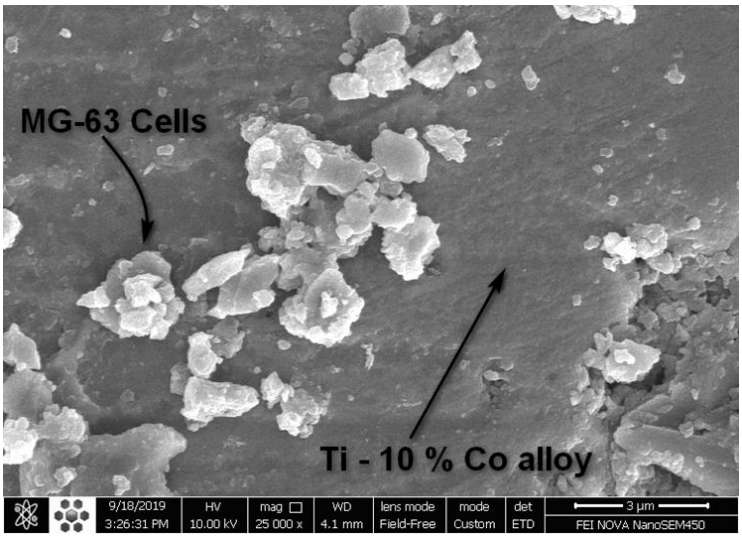

Figure 17. proliferation of MG63 osteoblast-like cells and the surface of sample (3), MG-63 cells were cultured on the surfaces for 2 days

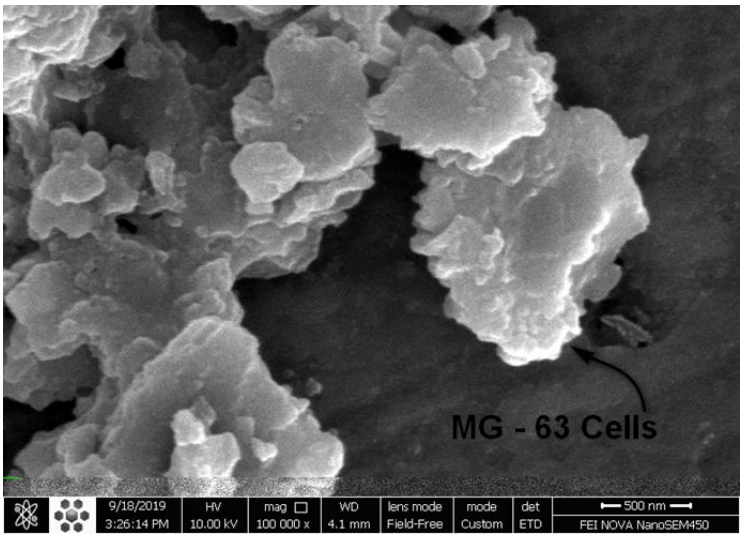

Figure 18. high magnification shows proliferation and cell adhesion of MG63 osteoblast-like cells. - MG-63 cells were cultured on the surfaces for 2 days for sample (3).

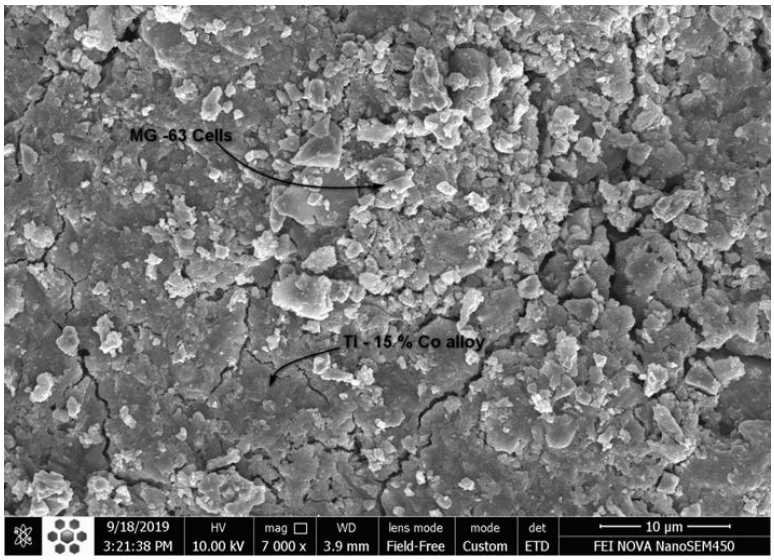

Figure 19. proliferation of MG63 osteoblast-like cells and the surface of sample (4), MG-63 cells were cultured on the surfaces for 2 days.

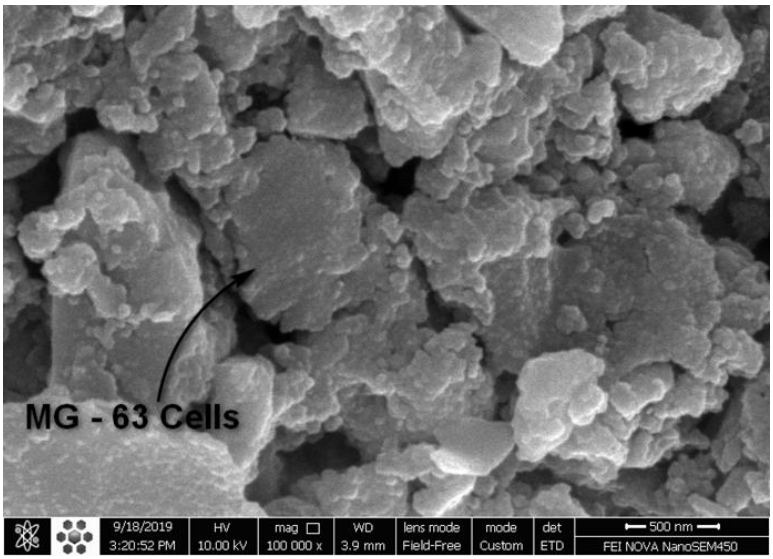

Figure 20. high magnification shows proliferation and cell adhesion of MG63 osteoblast-like cells. - MG-63 cells were cultured on the surfaces for 2 days for sample (4).

Cytotoxicity and Cell Viability

The three-dimensional Cell growth of human MG63 fibroblast cultures was observe by mitochondrial dehydrogenase activity (MTT-assay) in (3, 7, 14) days exposure periods. The results show that the cobalt addition expose considerable effect on cell viability, as have be seen in the MTT graphs, from figure (21) pure titanium alloys showed non cytotoxic effects due to an increasing in cell viability with time of exposure, which permit the inquiry of positive cell reaction with the titanium surface. Also Cell viability showing no sort of aggression result from titanium or cobalt materials, The benefit of cobalt additions compared with commercial pure titanium alloys, is the presence of active and non-cytotoxic compound (Ti_2Co) as showed in figure (22), On the other hand, use of powder technology as manufactured process showed an increase in the surface roughness which presents several attractive features, make the surface more attractive to the bone. From MTT graphs it was observed that the titanium and titanium-cobalt alloys surface became more active after 7 days of exposure as monitored in cell viability columns. Figure (23 and 24) at 10% and 15% cobalt showed remarkable increase in cell viability compared with pure titanium and Ti- 5% Co because of the increasing in a bio active intermetallic compound (Ti_2Co) at the alloy surface that allows Cell growth in the three dimensional very fast in the same period of exposure.

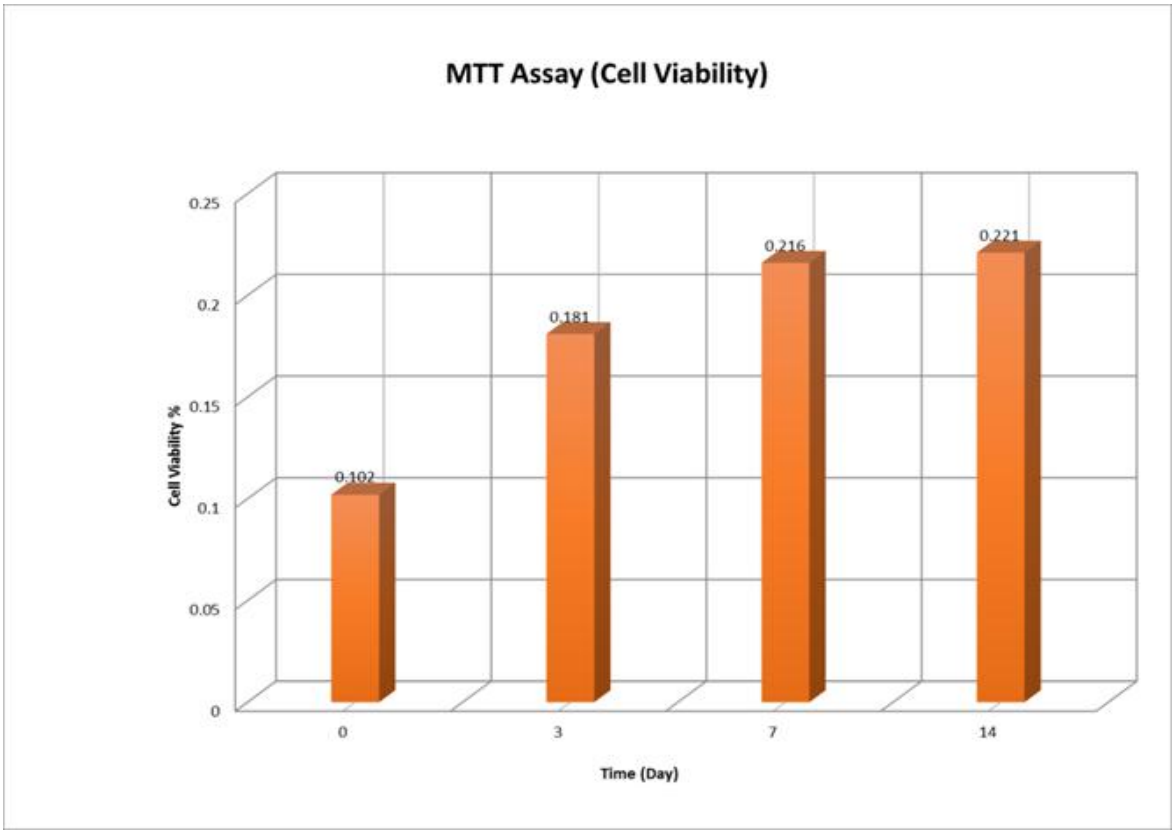

Figure 21. cell viability after (3, 7, 14) days exposure periods of sample (1).

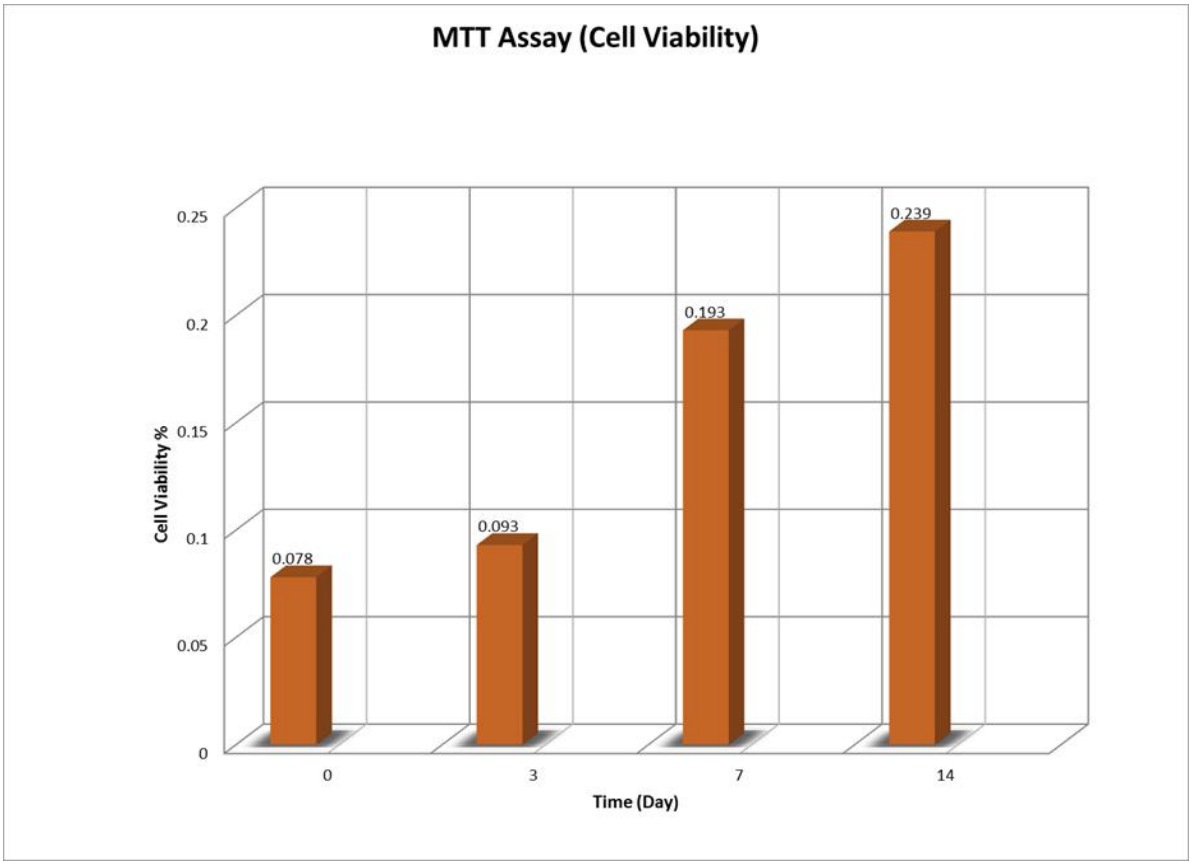

Figure 22. cell viability after (3, 7, 14) days exposure periods of sample (2).

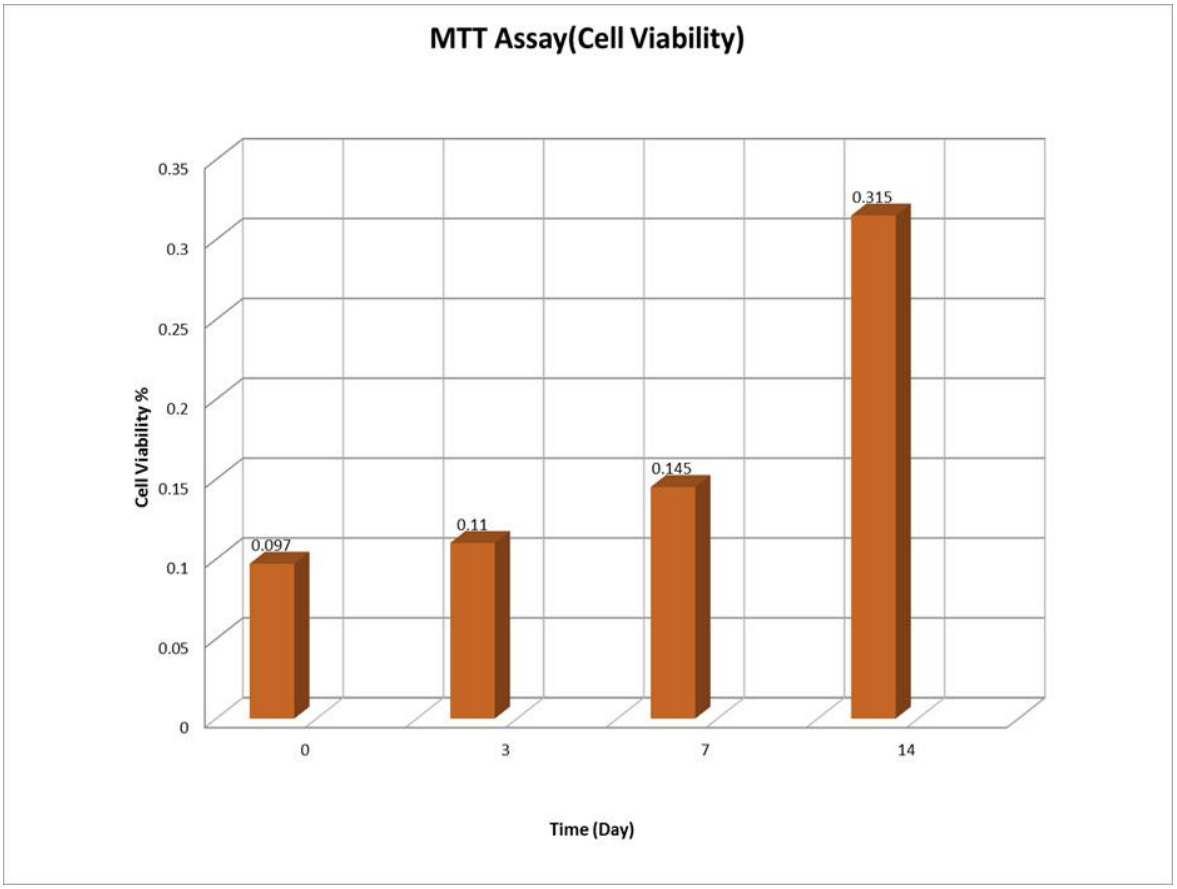

Figure 23. cell viability after (3, 7, 14) days exposure periods of sample (3).

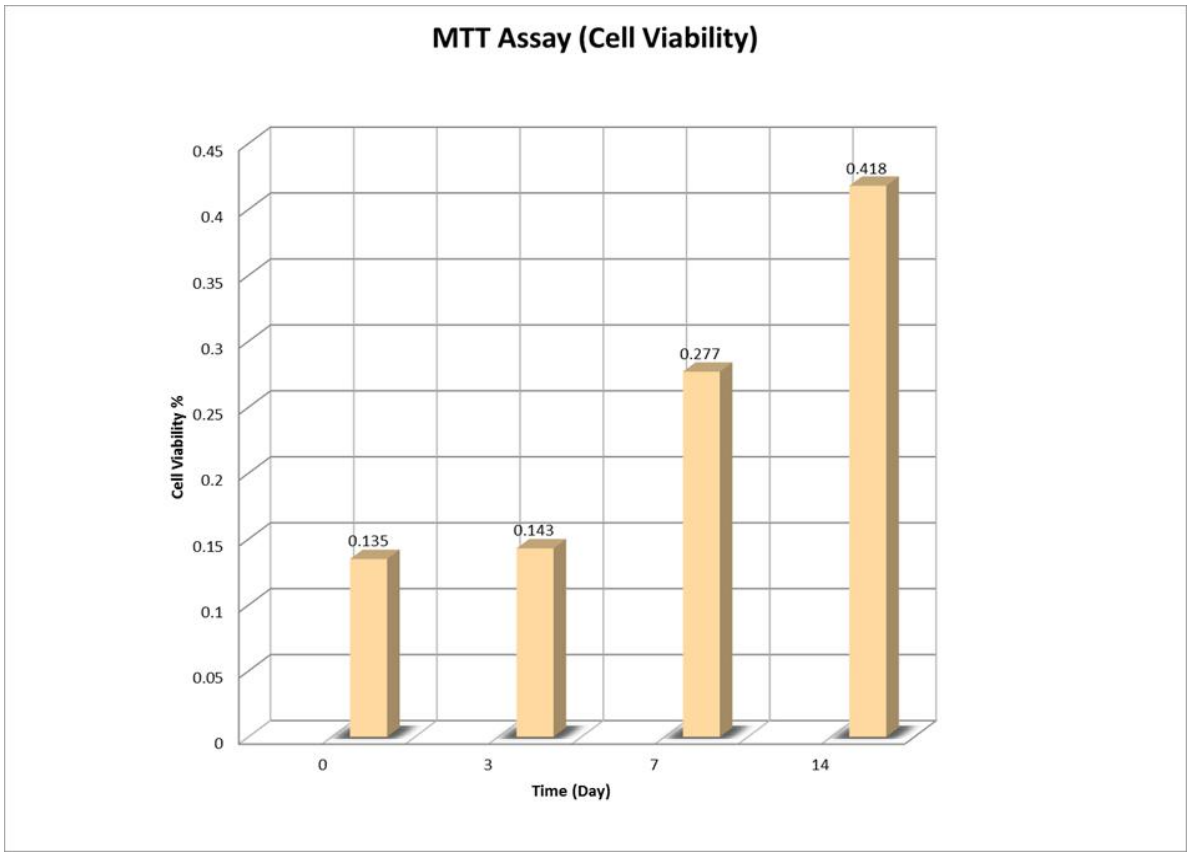

Figure 24. cell viability after (3, 7, 14) days exposure periods of sample (4).

Conclusion

It has been shown that all tested samples result in good positive response to MG63 osteoblast-like cell activity on their surface. The Biocompatibility MTT assay with MG-63 Cells demonstrate that cell attachments and cell viability showing no sort of aggression result from titanium or cobalt materials, The benefit of cobalt additions, when compared to commercial pure titanium alloys, is the presence of active and non- cytotoxic compound (Ti_2Co) which presents several attractive features, make the surface more attractive to the bone.

REFERENCES

1. Steinemann, S.G. and Perren, S.M. (1985).” Titanium as metallic biomaterial, in Science and Technology” (eds G. Lütjering, U. Zwicker andW.Bunk), DGM Oberursel, pp. 1327–1334.
2. Okazaki, Y. and Ito, Y. (2000) New, Ti alloy without Al and V for medical implants.Adv. Eng.Mater, 2 (5), 278–281.
3. Okazaki, Y. and Ito, Y. (2000). “New Ti alloy without Al and V for medical implants”,Adv. Eng.Mater, 2 (5), 278–281,
4. Ewald, A. and Thull, R. (2006).” Surface structure/electric field dependent protein adsorption” |, Biomaterialien, 3, 146,
5. Spyrou P, Papaioannou S, Hampson G, Brady K, Palmer R, McDonald F. (2002). "Cytokine release by osteoblast-like cells cultured on implant discs of varying alloy compositions", Clin Oral Implants Res 13: 623-30,
6. Al-Hiyaset AS, Bashabsheh OM, Darmani H. (2003). " An investigation of t cytotoxic effects of dental casting alloys", Int J Prosthodont 16: 8-12,
7. Ong JL, Cardenas HL, Cavin R, Carnes DL Jr. (1997)." Osteoblast responses to BMP-2-treated titanium in vitro". Int J Oral Maxillofac Implant; 12:649–54.
8. Erlebacher A, Filvaroff EH, Ye JQ, Derynck R. (1998). " Osteoblastic responses to TGF-b during bone remodeling", Mol Biol Cell;9:1903–18,
9. Tardif F, Ross G, Rouabhia M. (2004). "Gingival and dermal fibroblasts produce interleukin-1b converting enzyme and interleukin-1b but not interleukin-18 even after stimulation with lipopolysaccharide ,”J Cell Physiol 198: 125-32,
10. Alhayani B., H. Ilhan. (2020). “Image transmission over decode and forward based cooperative wireless multimedia sensor networks for Rayleigh fading channels in medical internet of things (MIoT) for remote health-care and health communication monitoring,” Journal of Medical Imaging And Health Informatics, vol. 10, pp. 160-168.
11. Alhayani B.,H.Ilhan. (2020). Efficient cooperative imge transmission in one-Way mult-hop sensor network,” International Journal of Electrical Engineering Education, pp. 1–17.
12. B. ALhayani, H. Ilhan, (2017). **“**Hyper spectral image classification using dimensionality reduction techniques**”,** International Journal of Innovative Research in Electrical, Electronics, Instrumentation and Control Engineering**,,** vol.5, pp.71-74.
13. B. .Alhayani, Milind Rane. (2014).” face recognition system by image processing” International journal of electronics and communication engineering & technology (IJCIET), vol.5, no.5, pp. 80–90.
14. B. Al-Hayani, H. Ilhan. (2020) “Visual Sensor Intelligent Module Based Image Transmission in Industrial Manufacturing for Monitoring and Manipulation problems,” Journal of Intelligent Manufacturing, vol.4, pp.1-14.
15. Ural AU, YÝlmaz MI, Avcu F. (2003). "The bisphosphonate zoledronic acid induces cytotoxicity in human myeloma cell lines with enhancing effects of dexamethasone and thalidomide", Int J Hematol 78:443-9,
16. Fatimah J. Al-Hasani. (2019). " Studying the effect of cobalt percentage on the corrosion rate of sintered titanium dental implants”, AIP Conference Proceedings 2190, 020011.
17. Fatima Al-Hassani. (2020)." Investigation of Ultrasonic Assisted Zirconia Coated Cp – Ti Alloys for Dental Implantation”, International Journal of Advanced Science and Technology, Vol. 29, No. 12s, pp. 1136-1143,