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Effect of ZrO_2 and Y_2O_3 Deposition on Biological Behavior of Ti-Base Alloys

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K E Y W O R D S

Ti-base Alloys, ZrO_2 and Y_2O_3 deposition, pack cementation process, biological behavior.

A B S T R A C T

Titanium has a good ability to attach to bone and living tissue, making it a perfect material for orthopedic implants. Because of the combination of high resistance to corrosion, biocompatibility and excellent mechanical properties. This work aims to study the Modifications of various base titanium implant samples producing by using powder technology (Ti-pure, Ti-45 % Ni, Ti10 % Co, and Ti-30 % Ta) by deposition of Nano Zirconia and yttria powders (70 % ZrO_2 and 30% Y_2O_3). Chemical pretreatment carried out to prepare the implant surface before deposition, while the deposition process accomplished by pack cementation. The Characterizations of samples accomplished before and after the surface treatment, which includes: microstructure observation, x-ray diffraction (XRD), MTT Assay (cell viability) and MTT assay (cell adhesion). From the SEM All samples Show that Nano Zirconia and yttria were homogeneously put on the surface and fully covered it which resulted in a substantial modification in surface morphologies. From XRD patterns the peaks slightly shifted to the low angle side also amorphous behavior was observed. From MTT graphs it was found that the titanium alloys surface after pack cementation became more active after 3 days of exposure in MG-63 cells and there was a remarkable increase in cell viability and cell attachment compared with untreated samples.

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1. INTRODUCTION

Titanium and its alloy are Lightweight, Strong, Corrosion Resistant, Non-toxic, Cost-efficient, Long-lasting, non-ferromagnetic, Biocompatible (not toxically and not dismissed by the body), Osseointegrated (the connection of bone with the implant), Long-range availability, Flexibility and elasticity are comparable with that of human bone. Most of us have knowledge of someone who

wanted orthopedic surgery to replace a damaged hip socket, joint of the leg, or badly broken bone. It is likely that medical-grade Ti was the preferred material for the surgeons to recreate these body parts. Natural Ti properties make it an ideal alloy to be used inside the body [1]. Biomaterials are defined as artificial or natural materials that are used to restore and replace the biological structure to recover its form and function in order to improve the quality and longevity of human life. By altering the surface feature of the titanium alloy through various methods, it is possible to change the surface texture for better hard and soft tissue interactions with the biomaterial. This improves the biocompatibility and survivability of the device and improves direct anchoring of these devices to the bone [2]. In general, the materials and architecture of the medical implant have two major mechanical demands. Under the yield strength of the materials, care stresses will be secure, and in cyclic loads, the stress of care will be kept under the fatigue limit. Due to a certain temperature made the mechanical properties of Ti-Ni depend on its phase state. In general, completely austenitic Ti-Ni material has sufficient properties for surgical implantation [3]. Ti-Ni (SMA) has formed a good material for medical uses as a result of its large corrosion resistance and shape memory efficiency. Such as use in orthopedic devices, internal broken bone fixation plates, dental arch orthodontic cords and maxillofacial oral surgery [4]. The Ti-Co device alloys are of both scientific and technical importance, Due to their possible uses as biomedical materials, in particular for hard tissue replacement HTR such as teeth and skeletons, Because of its higher body fluid resistance and biocompatibility, cobalt has also been used in medical implant alloy in dentistry and medicine during many years, the addition of cobalt improves the corrosion resistance of titanium, and its mechanical properties [5]. As the large important biomedical materials, the Ti-30%Ta alloy has gotten extensive use. In this research, titanium (Ti) alloyed with tantalum (Ta) will be considered because it offers significantly enhanced mechanical properties that include fracture toughness and workability, viewed Enhancement both pure Ti and pure Ta [6]. The behavior of titanium alloy, when placed in the biological system, depends on its surface feature and properties. The goal of surface modification is to enhance the biomaterial bioactivity so that the biomaterials show a higher ability to induce apatite, which in turn leads to rapid osseointegration. Medical Bone implant surfaces make the site of contact with the lively medical tissue surrounding them and are for that is important for improving the biological execution of medical implants. Generally, Surface engineering involves alteration of a medical device's topographical (like roughness) and chemical (like coating) properties. Lastly, the advent of nanotechnology has extended the scope of micro- to nanoscale topographical modifications; make an effect on cells, biomolecules and nanoscale ions. The mechanical properties of Ti are preserved by surface modifications by the deposition of materials, but the functionality of the medical implant surface can be enhanced by applying biochemical components that serve as indicators of improved medical bone renovation [7]. Diffusion coating is a surface modification process wherein the coating species are diffused into the substrate surface to form a protective layer [8]. The pack cementation process is also essentially a chemical vapor deposition process. The components to be coated are placed in a sealed or semi-sealed container (retort) together with a powder mixture that consists of metal elements to be deposited, halide activators and inert fillers. The sealed container is then heated under a protective atmosphere of argon and held for a specified duration, at elevated temperatures [9]. Zirconia is a transition metal zirconium, polycrystalline ceramics dioxide. Zirconia has favorable biological characteristics, add to excellent mechanical properties: (Lower potential for corrosion, getting lower cytotoxicity, Lower bacteria adhesion and Stress bearing positions where many alloys challenge its strength). Efficient metallic oxide dopant has different metallic oxide, including (MgO, CaO or Y_2O_3) by combining Zirconia with relatively small quantities of another metallic oxide, referred to as dopand [10]. They have been commonly studied because of the desirable properties of nanoparticles due to their useful surface area. Nano yttria stabilized Zirconia n-YSZ is a ceramic that has been widely examined in recent years. The response time is shortened by particles with lower grain size and a big specific surface. For that, in contrast with the bulk (YSZ), the (n-YSZ) method exhibits dissimilar behavior [11].

2. EXPERIMENTAL PROCEDURE AND MATERIALS USED

The experimental work introduces the method by which the samples were prepared, in other hands the characterization process and the types of experiments that were made in this work. The experimental work involves two main steps, the first step being to use the powder metallurgy

technique to prepare the four master samples (Ti-pure), (Ti-45%Ni), (Ti-10%Co) and (Ti-30%Ta). Titanium, nickel, cobalt and tantalum powders are the elementary powders used in the preparation of the master alloy. Table I display the properties of the powders and the groups of alloys shown in Table II.

TABLE I: Properties of powders

Element	Color	Shape	Mesh	Radius
Ti	Gray	Spherical	200	70 µm
Co	Black	Spherical	200	30 µm
Ni	Silver	Spherical	200	50 µm
Ta	Gray	Spherical	200	45 µm

TABLE II: Implant sample groups

Alloy	Group before coating	Group after coating
Ti- pure	P	P1
Ti-45%Ni	N	N1
Ti-10%Co	C	C1
Ti-30%Ta	T	T1

The material powder was weighted after selecting the weighted percentages for each sample using the sensitive balance type (sokg), where the total weight of each sample is 5 gm. The powder of each sample is mixed in a ball mill type (CAPCO) for 15 min. at 70 rpm speed for each sample in order to obtain a homogeneous and good mixing between components. The powder compaction process of each sample is carried out with the punch and die (made of D2 tool steel) with a die diameter of 15 mm as shown in Figure 1 and with the type of hydraulic pressing machine (KPD-50E), the powders were pressed for 10 min under the pressure of 7 ton and then samples with the same diameter of 15 mm and height of 5 mm were produced for each kind of alloy.

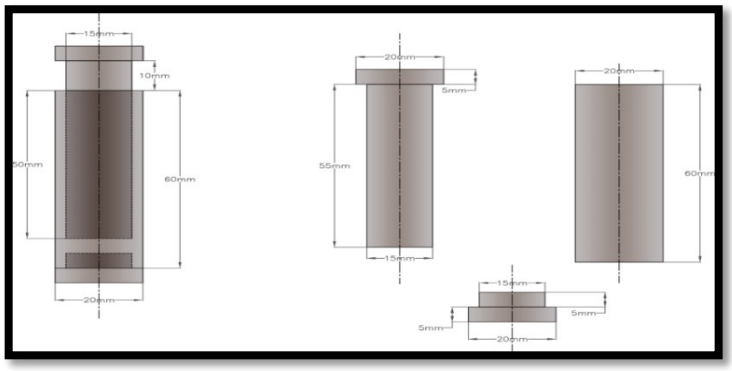

Figure 1: The die design shape

The sintering process was carried out for 3:30 hours by using the CVD quartz tube furnace at temperature 1000 °C under vacuum to prevent oxidation of the samples. The resulting samples with a diameter of 15 mm were transformed into a pin shape with a diameter of 3 mm after the sintering process by means of a wire cutting machine. Before chemical treatment, the ultrasonic surface treatment done by washing the surface of all dirt and impurities to achieve a successful treatment. The ultrasonic cleaner path type (KQ3200E) by the source of the ultrasonic wave used, where the samples were divided into groups according to their type of alloy. For one hour, the samples stay in the ultrasonic cleaner, and then they are removed and cleaned. Now the samples were ready for acid and alkaline treatment, which was used to eliminate oxide and contamination in order to achieve a clean and rough surface finish. The samples were submerged at 40 °C in HCl acid with a concentration of 0.5 mM for four hours, then washing the samples with ultrapure water and drying them. The samples were also submerged in NaOH for 24 hours at 60 °C with a concentration of 10

mM, then washed in ultrapure water and dried. The etching method was performed with the use of a water bath to obtain the etching temperature and maintain the samples at this temperature. After this stage, the samples were ready for the pack cementation process, where Zirconia, yttria and Sodium chloride as activator are the elemental powders used in this process, the percentage of powders is 95 %($Zr0_2$ with Y_2O_3) and 5 % NaCl and the total weight of deposition powders is 50 gm. The element powder was weighted using the Sensitive Balance Type (sokg) after selecting the weighted percentages for deposition powders, the powders are mixed in a ball mill type (CAPCO) for 30 min at high speed, this mixer used to have a homogenous distribution between the powders and to achieve good adhesion between elements. Finally, the alumina crucible filled with the resulted powder and master samples and then placed in the CVD quartz tube furnace at temperature 1000 °C for 3:30 hours under vacuum to avoid oxidation of the samples, this for carrying out the pack cementation process. The second step is the characterization of samples in order to obtain different sample characteristics, a number of tests have been performed. The tests (scanning electron microscope (SEM), X-ray diffraction (XRD), MTT Assay (cell viability) and MTT assay (cell adhesion)) were performed in order to verify the effect of various additions and deposition layer on titanium behavior, especially the biological behavior of all samples.

3. RESULTS AND DISCATION

I. Scanning Electron Microscope (SEM)

An electron microscope was conducted for scanning selected implant specimens (Ti-Pure, Ti-45%Ni, Ti-10%Co and Ti-30%Ta) whereas the image Microscopic observations were conducted for all groups of implant samples. This device utilized to provide acceptable surface topography observations and to define the influence of surface alteration on the surface textures of the medical implants. It is noted in Figure 2 for Ti-pure alloy, images of the surface before the deposition process and after the completion of the sintering process, where the surface has a high surface roughness as a result of the manufacturing process and this roughness is good and suitable for the purpose of increasing the efficiency of the deposition process. The same thing can be seen in Figure 3 which shows the microstructure of the Ti-45%Ni alloy. Notice that the surface roughness is less than other alloys; in addition to that, the surface porosity is less than other Ti alloys due to the size differences gap between the raw powders. Figure 4 represents the surface image of the Ti-10%Co alloy. This sample has a greater porosity than the rest of the previous alloys because there was a large difference in the particle size between the Ti powder and the Co powder, which led to the appearance of high surface porosity and a relatively rough surface. Finally, Figure 5 represented the Ti-30%Ta alloy, also notice that this sample has a good surface roughness resulting from the process of manufacturing by using the powder technology. Notice after the samples were deposited by Nano Zirconia and yttria using pack cementation that the surface morphology changes completely and have a new surface layer form on the samples and the deposition layer that was observed in nm scale as shown in Figures 6, 8, 10, and 12 proved that the Nanoceramic deposition layer is produced on the surface of the implant. In Figure 7 for the Ti-pure alloy, notice that the deposition layer is clear from the change in the surface microstructure and morphology compare to an image before the deposition process. As for the Ti-45%Ni alloy, it is shown in Figure 9, and as a result of little porosity and roughness, we obtained a layer of precipitation less than the rest of the alloys. While it appears in Figure 11 for Ti-10%Co alloy, that the deposition layer has occurred and will observe growth in a certain direction of the deposition layer as a result of large pore size, high porosity and good surface roughness that led to this distinct deposition behavior. Finally, notice that the Ti-10%Ta alloy shown in Figure 13. Deposits occurred on the surface of the alloy by making changes in the morphology of the surface. All samples Show that Nano Zirconia and yttria produced homogeneously on the sample surface and fully covered, resulting in a high modification in the surface morphology. This is due to the initial surface roughness that resulted from the manufacturing process (powder technology) and the primary surface treatment (acid-alkaline etching) that was done before the depositing process to prepare the surface for the deposition process. It was appeared surface utilizing pretreatment and powders manufacturing as the main process to have both homogeneous and adherent deposition layers was useful and successful.

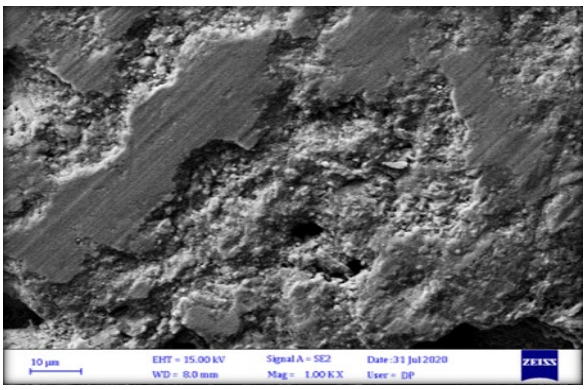

Figure 2: SEM image for sample (P).

Figure 3: SEM image for sample (N).

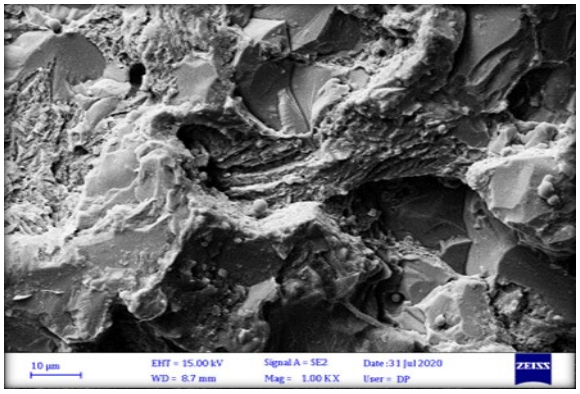

Figure 4: SEM image for sample (C).

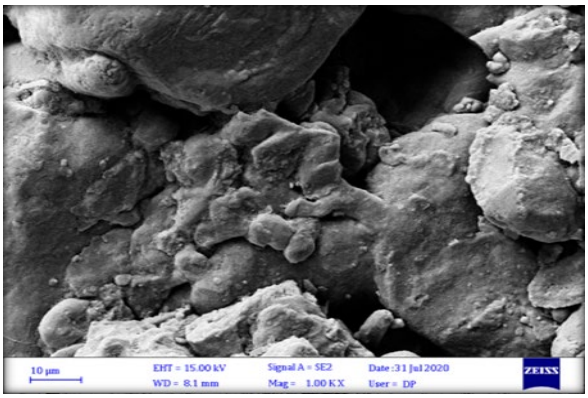

Figure 5: SEM image for sample (T).

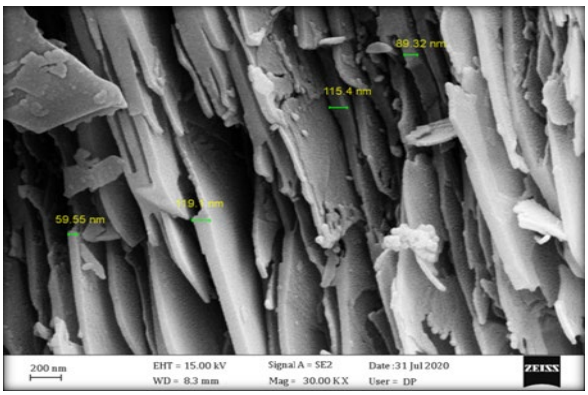

Figure 6: FE-SEM for sample (P1) in Nanoscale.

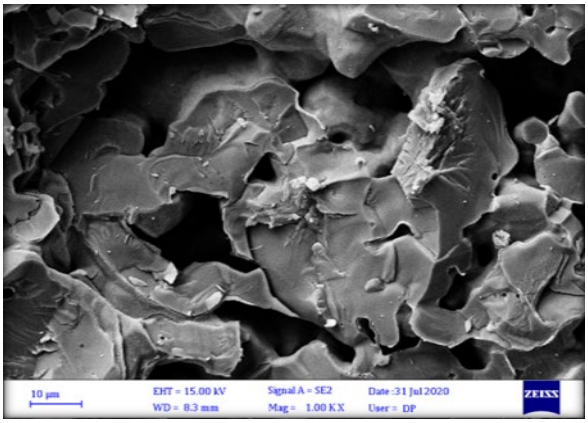

Figure 7: SEM image for sample (P1).

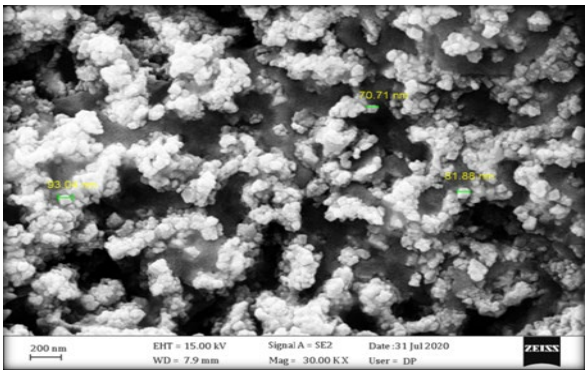

Figure 8: FE-SEM for sample (N1) in Nanoscale.

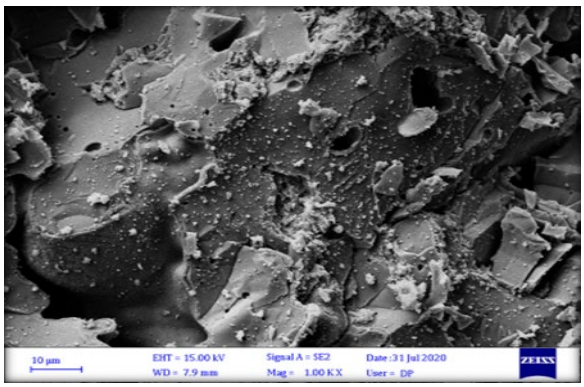

Figure 9: SEM image for sample (N1).

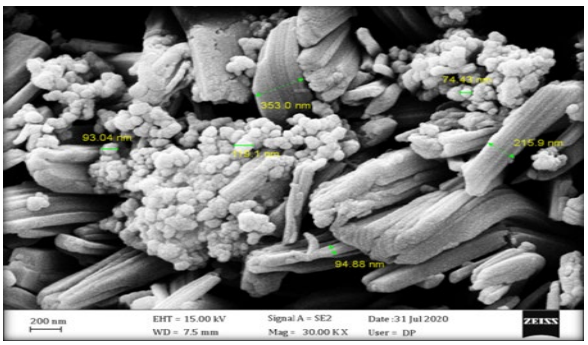

Figure 10: FE-SEM for sample (C1) in Nanoscale.

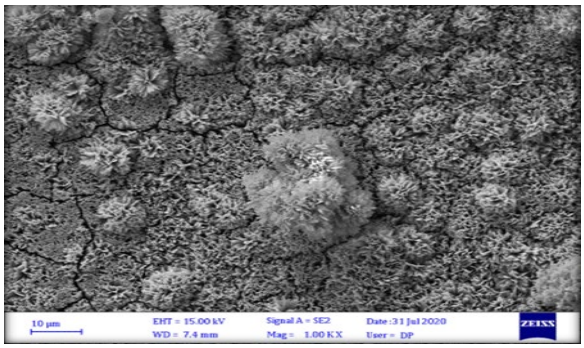

Figure 11: SEM image for sample (C1).

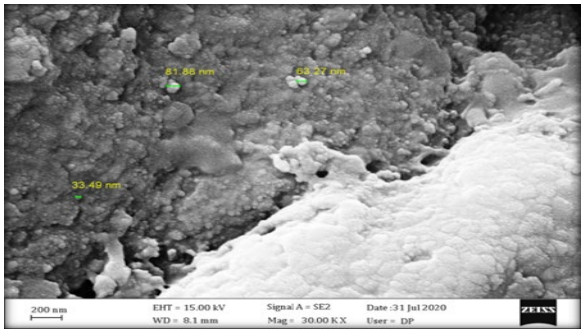

Figure 12: FE-SEM for sample (T1) in Nanoscale.

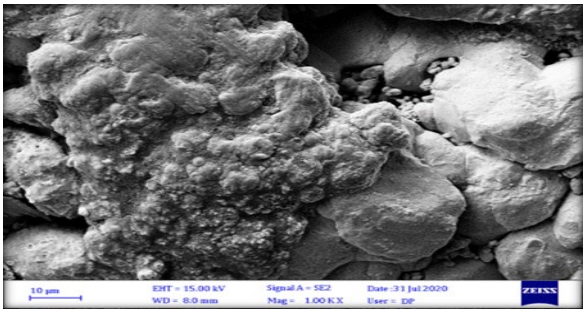

Figure 13: SEM image for sample (T1).

II. X-Ray Diffraction (XRD***)***

The XRD test was conducted by utilizing, Shimadzu X-ray diffract meter (type XRD - 6000) operated at 40 kV and 30 mA with Cu K α 1 radiation. In order to find out the structure and identify the phases of each sample, X-ray diffraction tests were done for all samples after the sintering and deposition practices. The diffraction patterns gained for the samples were the phases that developed as a result of sintering and deposition could be detected. Figure 14 refers to a pure titanium alloy with a single α phase. There are likely no presents of pure metals that prove the time and temperature of sintering utilized in this work result in full sintering reactions. Figures 15-17 illustrated the XRD

patterns of titanium with different alloying elements before deposition, Figure 15 included the addition of 10% Co to pure titanium the figure comprises intermetallic compound Ti_2Co in addition to the α-Ti phase. Whereas Figure 16 showed the XRD pattern of titanium with 45% Ni, it was noted that the addition of nickel leads to form an intermetallic compound (Ni_2Ti_2) in addition to the α-Ti phase due to the titanium – nickel phase diagram. Figure 17 showed the effect of tantalum addition to the pure titanium powder and from this figure, the β second phase appeared in addition to the-titanium phases, from XRD patterns only Ti-30% Ta consist mainly of two phases (α + β) biphasic structure. Figures 18-21 illustrated the XRD patterns of samples after (ZrO_2 andY_2O_3) deposition by the pack cementation process. It is obvious that Amorphous behavior was observed in the XRD after deposition nearly at 2θ (15.799) for all samples, the peaks in the XRD pattern for titanium alloys matched the diffraction angles of α titanium well. But With (ZrO_2 andY_2O_3) deposition, the peaks slightly shifted to the low angle side. There was no indication that other phases included in any of the present diffraction patterns.

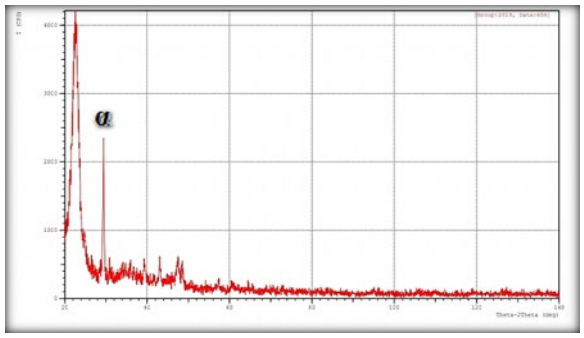

Figure 14: XRD pattern of the sample (P).

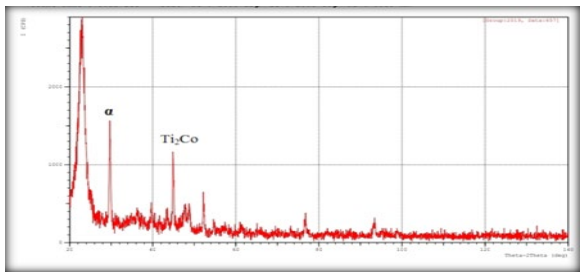

Figure 15: XRD pattern of the sample (C).

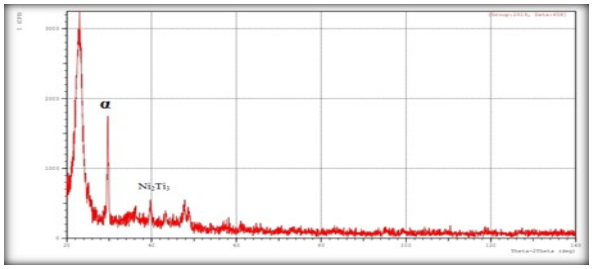

Figure 16: XRD pattern of the sample (N).

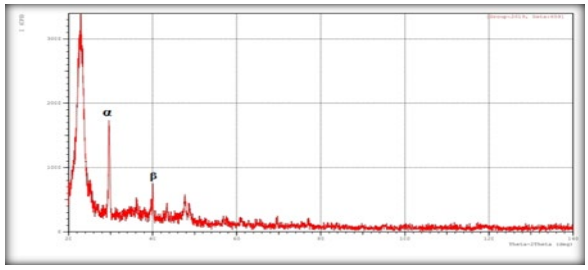

Figure 17: XRD pattern of the sample (T).

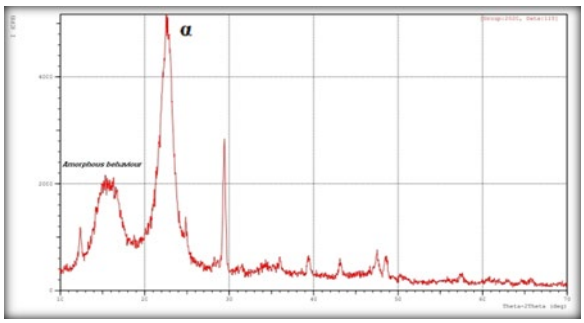

Figure 18: XRD pattern of the sample (P1).

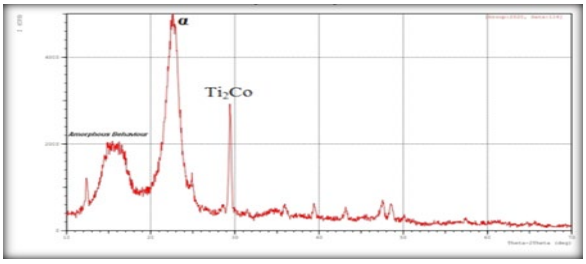

Figure 19: XRD pattern of the sample (C1).

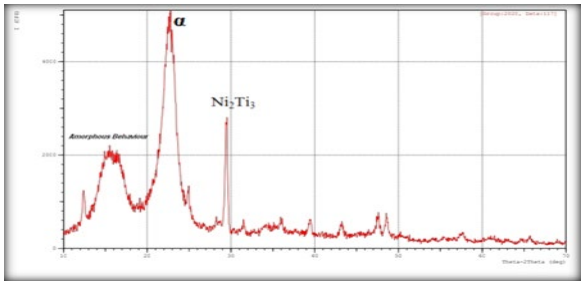

Figure 20: XRD pattern of the sample (N1).

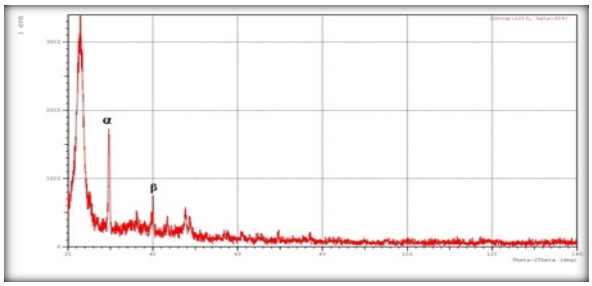

Figure 21: XRD pattern of the sample (T1).

III. MTT Assay (Cell Viability)

The three-dimensional cell growth of human MG63 fibroblast cultures was observed by mitochondrial dehydrogenase activity (MTT-assay) in (24, 48, 72) hours of exposure periods. The results show that the Ti alloys expose a considerable effect on cell viability, as have been seen in the MTT graphs, from Figure 22 titanium alloys showed an increase in cell viability with a time of exposure this proved that titanium is medical materials with high biocompatibility and used widely in the production of the medical implant, also it not make any bad effect on human cells, which permit the inquiry of positive cell reaction with the titanium surface. Also, Cell viability showing no sort of aggression result from titanium or additional materials. The benefit of material additions compared with commercially pure titanium alloys is the presence of an active and non-cytotoxic compound, On the other hand, the use of powder technology as a 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 alloys surface is active after 3 days of exposure as monitored in cell viability columns. Where Ti-10%Co showed a remarkable

increase in cell viability compared with other titanium alloys because of the increase in a bioactive intermetallic compound (Ti_2Co) at the alloy surface that allows Cell growth in the three dimensional very fast in the same period of exposure.

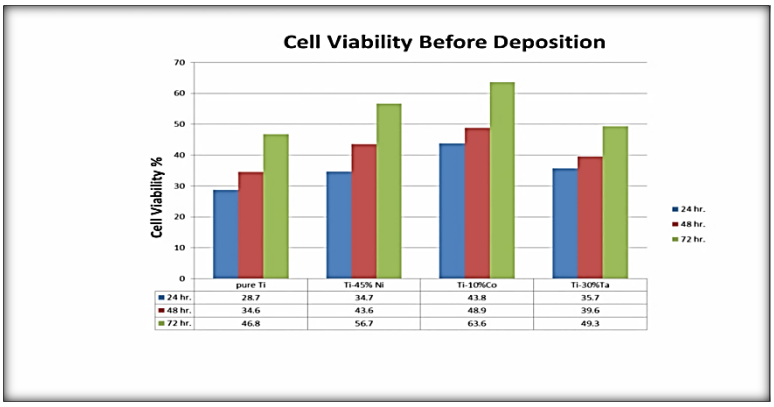

Figure 22: MTT assay (cell viability) of samples before deposition.

After the process of depositing a Nano-ceramic material on the surface, will notice in Figure 23 a complete change of surface properties such as the change in the chemical composition and morphology of the surface is happen, therefore the biological behavior of all samples has changed and the growth of cells has increased for several reasons, the most important of it which is the surface roughness resulting from the manufacturing process (powder technology) in addition to chemically treating titanium was to modify its surface topography and chemistry such that its apatite forming ability is improved. After alkali treatment in NaOH, apatite nucleation may occur when soaking in simulated body fluid by the complex process as found in Jonšov L. [12]. The thickness of the precipitated apatite film has been found to rise always over time, and Ti pretreatment with HCl and next with NaOH treatment begin to be an effective process to boost the capacity of surface medical bone bonding. The deposition of Nano-Zirconia and yttria, which are original biomedical materials on the surface, which contributed to the rising of the surface's biological activity, will notice little difference in cell viability between alloys due to different surface properties such as hardness, porosity, and surface roughness. It is noted that Ti-10%Co alloy has the highest value for cell abundance and that is due to the fact that titanium and cobalt are medical materials with high biocompatibility, And the large pore size led to the amount of powder deposited on the surface more than the rest of the alloys Thus, make the amount of bio-sedimentation layer was more, which helped to increase the viability of the cells and more effective than other Ti alloys.

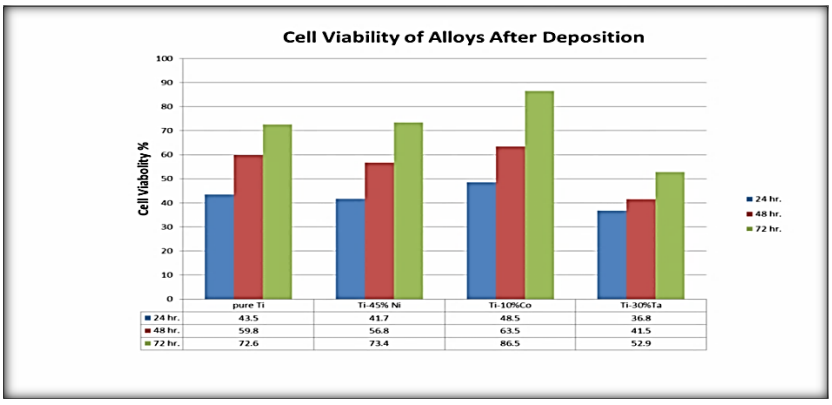

Figure 23: MTT assay (cell viability) of samples after deposition.

IV. MTT Assay (Cell Adhesion)

The experiment was to determine how well cells grew (proliferated) on the surface of samples and the adhesion of cells on different titanium alloys. From images, proliferation and cell adhesion of MG-63 osteoblast-like cells were cultured after 3 days and prepared for electron microscopy. Cells grown for the titanium alloys as in Figures 24- 27 possess attachment of cells is found in all alloys, but with little different, that the high adhesion cells are shows in Figure 26 for Ti-10%Co alloy due to

have good surface roughness and big pores size and high porosity percentage this make attachment of bone cells is better than other alloys.

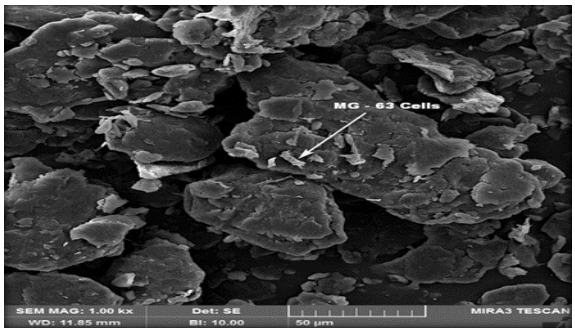

Figure 24: MTT assay (cell adhesion) of the sample (p).

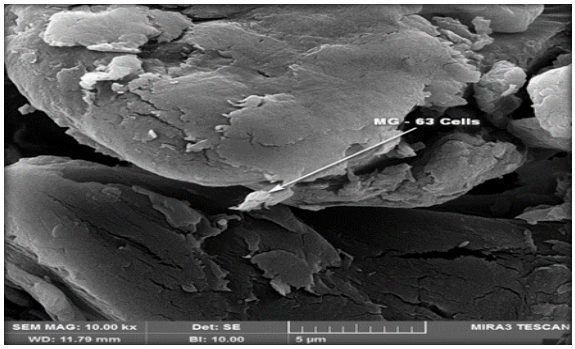

Figure 25: MTT assay (cell adhesion) of the sample (N).

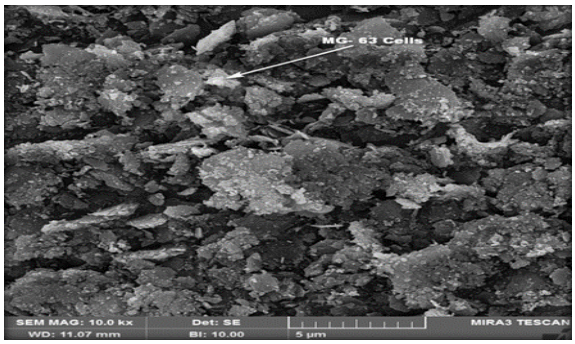

Figure 26: MTT assay (cell adhesion) of the sample (C).

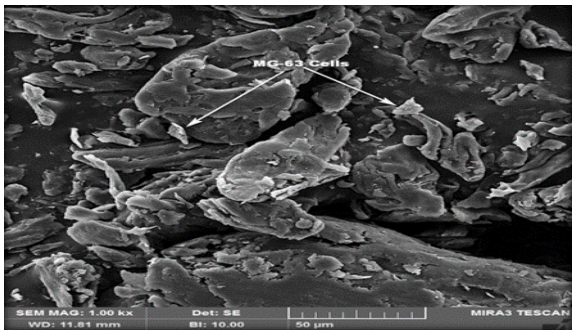

Figure 27: MTT assay (cell adhesion) of the sample (T).

Showed in Figure 28- 31 for titanium alloys after depositing by Nano Zirconia and yttria, will be observed that the cell attachment is the increase for all alloys with few differences between them due to the difference in the surface properties such as morphology, porosity and surface roughness, so notice in Figure 30 for Ti-10%Co alloy that have the better cell adhesion compared to other alloys because of the high deposition of bioceramic material that occurs due to high porosity that has on its surface. Finally, we found that the manufacturing process with the powder technology gives good

initial porosity and surface roughness for the growth of bone tissue, in addition to the surface preparation process, chemical treatment to obtain a clean surface, additional surface roughness, and to have a NaOH layer to increase the bio-activity of alloys, at the last, a medical Nanoceramic layer is deposited on the surface of all alloys and make a large increase in growth and attachment of cells.

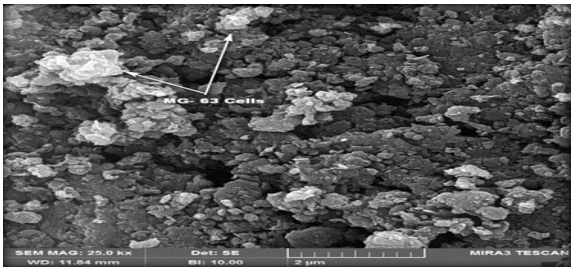

Figure 28: MTT assay (cell adhesion) of the sample (p1).

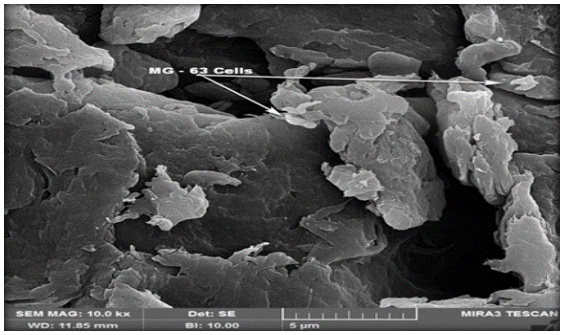

Figure 29: MTT assay (cell adhesion) of the sample (N1).

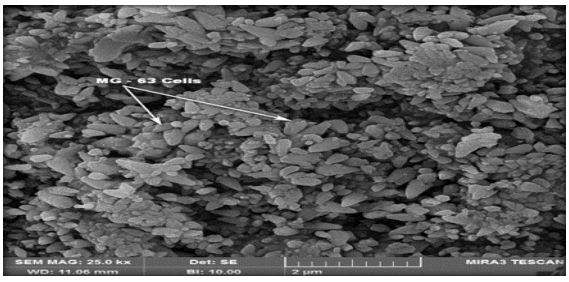

Figure 30: MTT assay (cell adhesion) of the sample (C1).

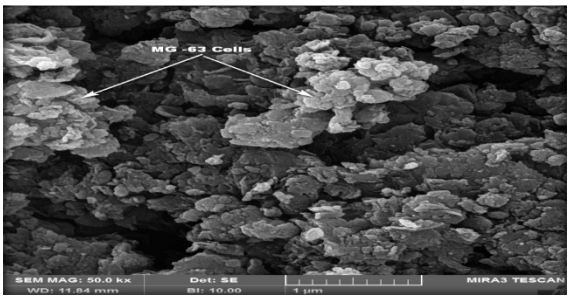

Figure 31: MTT assay (cell adhesion) of the sample (T1).

4. CONCLUSION

The use of pack cementation as a Nanoceramic deposition process resulted in the formation of Nano Zirconia and the yttria layer on the surface, also using pre-surface treatment was beneficial and successful to achieve homogenously and adhere depositing layer. From the SEM, all samples showed that Nano Zirconia and yttria were homogeneously distributed on the surface and completely covered which resulted in a significant change in surface morphologies. This was due to the initial surface roughness that resulted from the manufacturing process (powder technology) and primary surface treatment (acid-alkaline etching) that was done before the depositing process to prepare the surface

for the deposition process. From XRD patterns the peaks in the XRD pattern for titanium alloys matched the diffraction angles of titanium well. But after the pack cementation process, the peaks slightly shifted to the low angle side also amorphous behavior was observed in the XRD after deposition nearly at 2θ (15.799) for all samples, there was no indication that other phases included in any of the present diffraction patterns of samples after (ZrO_2 andY_2O_3) deposition. From MTT graphs it was found that the titanium alloys surface after pack cementation became more active after 3 days of exposure in MG-63 cells and there was a remarkable increase in cell viability and cell attachment compared with untreated samples.

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