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DEVELOPMENT OF NANO-CERAMIC-TiO_2+AL_2O_3LAYERS FOR INCREASING ANTIBACTERIAL EFFECT ON PROSTHETIC IMPLANT

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ABSTRACT

The infection of the titanium implants still remains a problem which is usually difficult to treat and may lead to eventual implant removal. As a result, preventive measures are necessary to mitigate implant-related infection; one important strategy is to render the implant surface antibacterial. The objective of the present study was to develop a biomedical material for preventing and reduction the effect of *Escherichia coli* bacteria type on the prosthetic implants; this was done by investigating the effect of the radioisotope with CO^{60} as the source of Gamma ray with thickness film of metal oxide nanoparticles (NPs) of (Alumina and titanium) coated on Ti6Al4V alloy by using RF magnetron sputtering for growth-inhibitory effect on *Escherichia coli* bacteria. The TiO_2 coated as mid layer between substrate Ti alloy and $Al_2O_3$to increase the adhesion between coated film and substrate, also to reduce thermal expansion mismatch between substrate and Al_2O_3layer. The nanoparticles size of Al_2O_3 was380nm. The results refers that the increasing of the thickness (t) of coated exposure of Gamma ray lead to increase the absorption (A) of radiation and reflection (R). By increasing(R), the material of coated become as source for Gamma ray around environment to reduce the growth of bacteria. In this study, the Gamma ray and increasing thickness at very high concentrations Al_2O_3 layer with nanoparticle size380nm are contributed to reduce growth of *Escherichia coli.* The effect of inhibited bacteria increased with increasing thickness of thin film.

Keywords: Ti6Al4V alloy, TiO_2+ Al_2O_3 ceramic nanoparticles, RF sputtering, gamma ray, *Escherichia coli* bacteria.

1. INTRODUCTION

The implant-associated infections and bacteria effect has been one of the main serious complications in the prosthesis field. This challenge is further complicated by the concern over the development of antibacterial resistance as a result of using traditional antibacterial for infection prophylaxis. Titanium alloys are the key to biomedical materials and highly desirable for implant application because of their good and high biocompatibility, mechanical properties, and corrosion resistance. In implants made of titanium, the various surface modification techniques required because:

1. In implants made of titanium, the normal manufacturing steps usually lead contaminated surface layer that is surfaces are clearly not appropriate for biomedical applications and some surface treatment must be performed.

2. The specific surface properties that are different from those in bulk are often required. The difference between the modulus of elasticity of the metallic implant of Ti and the modulus of elasticity of bone tissue can be controlled with the fabrication of the structure surface of Ti implants[1].

Ceramic particles are biomaterial with general properties as (Al_2O_3, ZrO, HAp and TiO_2); they are widely used in medical and pharmaceutical application[2]. The surface modification techniques are a general concept that can be divided into: mechanical, chemical and physical methods. The physical methods refer to such methods such as, thermal spraying and physical vapor deposition (PVD), RF sputtering, laser or electrical discharge in vacuum, glow discharge plasma treatment and ion implantation. The ceramic particle advantages of using plasma RF sputtering technique for enhancing the properties of surface Ti alloy[3].For instance, the thermal expansion coefficient of Ti alloy substrate is 8.7×10^{-6}/K, for TiO_2 is 7.249×10^{-6}/K and Al_2O_3 is 8.2×10^{-6}/K. Thus, the addition of TiO_2 onto the substrate is expected to reduce thermal expansion mismatch between substrate and coated layers Al_2O_3[1]. The RF sputtering could convert the scattering particle to nano size. Nanoparticle size is very important in medical application because biological process takes place in nano scale and can poses address for most important biomedical problem[4].

Then one of most important and active new methods for reducing bacteria activity, antimicrobial is applications of nanotechnology, using particle or molecules with nano scale in medical application for killing or reducing the activity of numerous microorganisms such as silver, zing, aluminium and titanium oxide[5-6]. The photo-induced bactericidal activity in TiO_2coatings appear to be a promising, powerful approach in the fight against transmission of infectious diseases, that proposed byLivia V. *et al* [7]. Aluminium oxide with nano size is very important application in composites, biomaterial, reinforcement and absorbent[8-10]. Meixue Chen *et al*were investigated bacterial action of Al_2O_3, Ag/ Al_2O_3 and AgCl/ Al_2O_3 on

pure culture of *Escherichia coli* K12[11]. Simon D. A. *et al* were studied *Escherichia coli* MG1655 exposed to TiO_2,Al_2O_3 nano particles, or multi walled-carbon nanotubes (MWCNT) used $Al_2O_3$with 11 nm size reduce bacteria *E. coil* spaces 35%, 70%, and 68% at 10,100, and 500 µg/ml by Dos–depended, particle penetration proposed mechanism, the resolute show that nanoparticles toxicity depends on their chemical composition, size, surface charge, and shape but not on their crystalline phase. *E coil*,*B* subitils,*P* florescence exposed to $Al_2O_3$with 60 nm size reduces bacteria spaces 36%, 57%, and 70% at 20mg/ml flocculation proposed mechanism[12].Jiang W.*et al* were investigated toxicity of nano-scaled aluminium, silicon, titanium and zinc oxides to bacteria (Bacillus subtilis, *Escherichia coli* and Pseudomonas fluorescence)toxicity of released metal ions was differentiated from that of the oxide particles. ZnO was the most toxic among the three nanoparticles, causing 100% mortality to the three tested bacteria. Al_2O_3 nanoparticles had a mortality rate of 57% to B. subtilis, 36% to *E. coli*, and 70% to P. fluorescens. SiO_2 nanoparticles killed 40% of B. subtilis, 58% of *E. coli*, and 70% of P. fluorescens. The analysis results show that the toxicity was affected by bacterial attachment [13].Jeng HA and Swanson J. were investigated the TiO_2, ZnO, Fe_3O_4, Al_2O_3, and CrO_3 with particle sizes ranging from 30 to 45 nm mitochondrial function, cellular morphology, permeability of plasma membrane, membrane leakage of lactate dehydrogenase (LDH) and apoptosis were assessed under controlled with exposed conditions (2 to 72 h of exposure). However, Fe_3O_4, $TiO_2$and Al_2O_3 had no measurable effect on the cells until the concentrations reached greater than 200 microg/mL, flow cytometer tests showed that apoptosis took place in cells exposed to ZnO nanoparticles. More cells became necrotic as the concentrations increased[14].

3. THEORY

The Gamma rays are electromagnetic radiation of nuclear origin with wave lengths in the region of 3×10^{-11} m to 3×10^{-13} m. It is more convenient to describe the radiation in terms of energy than in terms of wavelength since the energy absorbed from the radiation is basically of interest. The relationship between wavelength and energy is:

$$E = hc/\lambda \quad (1)$$

Where: h is Plank's constant, c is the velocity of light, and λ is the wave length. Substituting for the constants gives:

$$E(eV) = (1.24 \times 10^{-6}) / \lambda(m) \quad (2)$$

In terms of energy the wave length range 3×10^{-11} m to 3×10^{-13}mbecomes approximately 40KeV to 4 MeV. The Gamma ray is most widely method used for treatment the surface of sample. It is emitted by radioactive isotopes are either mono-energetic or have a small number of discrete energies, Cobalt-60(CO^{60}), for example, gives equal numbers of Gamma photons of energy 1.332 and 1.173 MeV. Exposure to Gamma ray irradiation is a frequent, clean, and superior method used to prevent bacterial contamination of sterilized biomedical end products. However, the potential damage induced by gamma ray irradiation of collagen is of concern because of the decay of bioactivity, which correlates with considerable structural alterations[16].Shielding of Gamma radiation primarily involves the interaction of Gamma radiation with matter via three main processes:(1) photoelectric effect,(2) Compton scattering, and (3) pair production. According to Lamber law and the relation between absorption (A), reflection (R) and transmission (T), if parallel beam of monoenergetic Gamma rays goes through material,the photon will go through thickness material an interaction is:

$$I = I_\circ \ e^{-\mu t} \quad (3)$$

Where: I is the intensity after shielding; $I_\circ$ is the incident intensity,µ:mass absorption coefficient (cm^2/g), t: thickness of absorber[17].From equation (3):

$$ln\frac{I}{I_\circ} = -\mu\, t \quad (4)$$

$$t\mu = 2.303\, log\frac{I_\circ}{I} \quad (5)$$

Where: the term $log\frac{I_\circ}{I}$ represent the absorption = A

$$\therefore \mu = 2.303\frac{A}{t} \quad (6)$$

So, from equation (6) at increasing the thickness of absorber t, the absorption A increasing and absorption coefficient µ decreasing.

$$R=1-T-A \quad (7)$$

From equation (7) at increasing A, the R increasing and T decreasing. Surface modifications aimed at obtaining antibacterial properties using RFsputtering Al_2O_3.

Escherichia coli(E.coli) are the name of a germ, or bacterium that lives in the digestive tracts of humans and animals. The *E. coli* is the most prevalent infecting organism in the family of gram-negative bacteria known as Enterobacteriaceae [18]. There are many types of E. *coli*, and most of them are harmless. But some can cause bloody diarrhea. Some strains of *E. coli* bacteria (such as a strain called O157:H7) may also cause severe anemia or kidney failure, which can lead to death. Other strains of *E. coli* can cause urinary tract infections or other infections. So that the treatment of this type of bacteria becomes very important[5].

3. MATERIAL AND METHOD

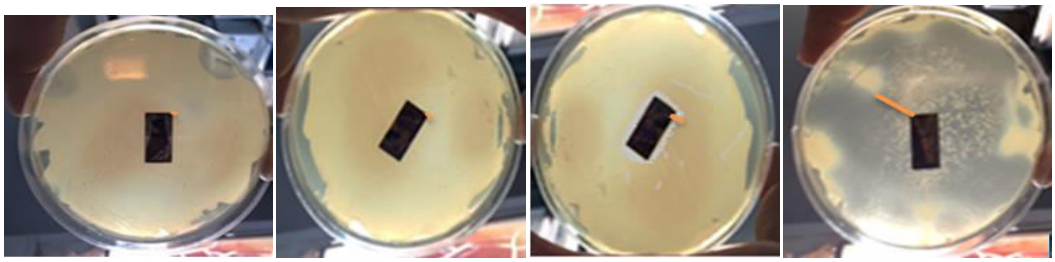
The purpose of this work is to study the growth inhibition effects of the ceramic which may be reflects on toxicity behaviour of $Al_2O_3$and TiO_2 on *E.coli* bacterial

species in the environment. The radioisotope with Colbalt-60 is used as the source for Gamma rays for a lot of medical applications because this source kills bacteria[15].According to the manufacturer, the titanium alloys (Ti_6Al_4V) GR2 ASTM F136 have a composition of Ti = 89.2, Al = 5.5-6.5, V = 3.5-4.5, Fe = 0.40, C = 0.1, N = 0.05, O = 0.20, H = 0.0125. The specimens of Ti6Al4V alloys were used as substrates in plasma RF sputtering system. The specimen alloys were grained with various grades of SiC paper and polished using Struers-DAP-U system, Denmark. The polished alloys were ultrasonically cleaned. Two types of targets were used: TiO_2 (4~5µm of particle size with a purity of 99.995%) and Al_2O_3 (1µm particle size with a purity of 99.999%)..The operation frequency of the RF generator was 13.65 MHZ with working pressure was 5.5×10^{-3}Torr and a vacuum chamber 1×10^{-7}Torr. Thin films were deposited on substrates Ti_6Al_4V alloy using TiO_2+Al_2O_3 targets. The TiO_2 was used as the first deposited layer on the substrates which was depend on working conditions of TiO_2, then under continues vacuum using Al_2O_3 target with same working condition of TiO_2, the second layer of Al_2O_3 was deposited film. The distance was fixed at (7cm) between target and substrates, substrate temperature (300°C), total time sputtering of TiO_2 is fixed at (2hr) while for Al_2O_3 change (4, 6 and 9 hr) to increase the thickness of thin film. The samples were annealed at (600°C) for (2hr) in a vacuum furnace. The multi-layer coatings comprised of TiO_2 and Al_2O_3 were deposited onto Ti_6Al_4Valloy.The samples cleaning using Gamma ray with irradiation at 2.5 t0 3 mega read using Gamma cells 220V with CO^{60} source and the energy use radiation was 1.25MeV with a dose rate 90.4 rad/min and 80 cm source to object distance.

The *E. coli* bacterial cells (DH5α strain) were used as the biological material because *E. coli* is the most common microorganism used as a model organism. The investigation of bacterial surface colonization was carried out under flow condition of culture medium. The samples were placed in a biological flow reactor chamber. An annular holder with holes was used to fix the samples. The rotational flow was forced by electromagnetic stirrer. The whole system with samples was steam autoclaved (Prestige Medical 2100 autoclave). At the next stage, the reactor was refilled with 200mL of sterile culture medium for 15min under pressure 121inch^2. The muller-hintonagar medium was composed of Pep ton G 0.5%; yeast extracts 0.5%, and NaCl 1.0%. The samples were incubated for 24 h in a medium containing a *E. coli* (DH5α strain) at 37^0C under flow condition. Observations were made to count bacteria adhering to the sample surfaces. One observation places were selected from each group of the studied samples. The microorganisms used in this study are medically significant E. *coli* opportunistic pathogens. *E. coli* was frequently associated with many types of infections. The anti-bacterial activity of the Alumina Al_2O_3nanoparticles was tested against bacteria.

4. RESULTS

The results of the present work includes the following tests, Figure-1 represents the bacteria growth on the Ti6Al4V alloy coated with thin film ceramic (TiO_2+Al_2O_3) the thickness of TiO_2 is fixed while Al_2O_3 change, as in (Figure-1a) low thickness film without any treatment,(Figure-1b, c, and d) treatment with gamma ray constant intensity but different thickness of thin filmAl_2O_3. At low thickness Al_2O_3Figure-1(a&b)very little effect inhibiter bacteria , the clear effect at increasing thin film (c), and at high thickness $Al_2O_3$the inhibiter bacteria increasing (d). The radius of area inhibiter bacteria increasing with increasing thickness of Al_2O_3 film.



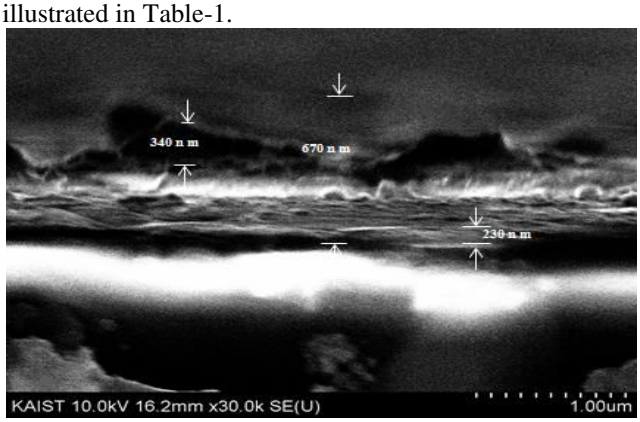
(a) (b) (c) (d)

Figure-1. Bacteria growth on the proposed alloy coated with thin film ceramic (TiO_2+Al_2O_3):(a) low thickness without any treatment,(b,c&d) treatment with gamma ray constant intensity and different thickness Al_2O_3film.Cross section SEM micrograph was use to determining the thickness of the thin film (Ti+Al_2O_3) coated on Ti-6Al-4V alloy.

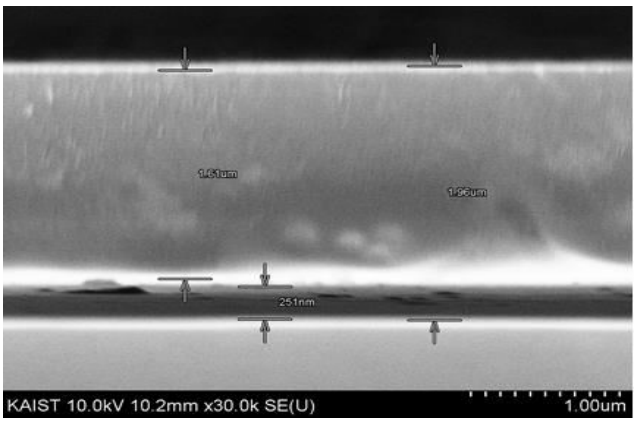
Table-1. Determination of the thin film thickness using SEM.

Figure	Thickness of TiO_2 layer	Thickness of Al_2O_3 layer	Total thickness (Ti O_2+Al_2O_3)
a,b	230 nm	340 nm	670 nm
c	245 nm	750 nm	920 nm
d	251 nm	1.61 um	1.96 um

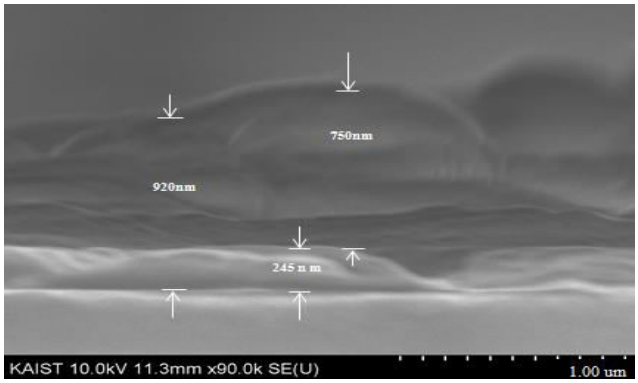
The thickness of TiO_2 film approximately constant in range (230-251nm) with constant time sputtering (2 hr) while Al_2O_3 film increasing (340nm, 750nm and 1.61um) with increasing time (4, 6 and 9 hr) respectively as shown in Figure-2 and illustrated in Table-1.



(a)&(b)



(d)



(c)

Figure-2. Cross section SEM micrograph of(Al_2O_3+TiO_2) coated Ti6Al4Valloy.

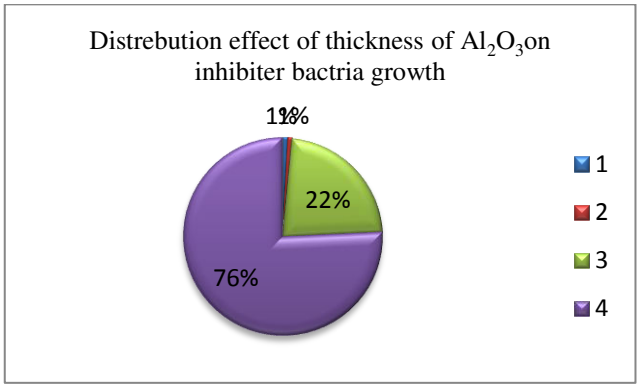


Figure-3. Distribution effect of thickness of Al_2O_3 layer on bacteria E. coli.

Figure-3 and results of Table-2 represent chart diagram for Figure-1, for same thickness film Al_2O_3 (340nm) the treatment or not with gamma ray not effect on radius area and percentage inhibiter bacteria growth. The radius area inhibiter bacteria growth increasing with increasing thickness of thin film Al_2O_3lead to increasing percentage inhibiter bacteria growth. Also the analysis results show that the metal oxide nanoparticles help to effect on the cells and decreasing the growth of bacteria. The plasma technique helped to create film with good properties affected on the bacteria E. coli.

Table-2. Distribution effect of gamma ray and thickness of Al_2O_3layer on bacteria.

No.	Total thickness of Al_2O_3 layer	treatment or not with gamma ray	Radius area inhibiter bacteria growth	Percentage inhibiter bacteria growth%
1	340 nm	no	0.1 mm	1
2	340 nm	yes	0.1 mm	1
3	750 nm	yes	3 mm	22
4	1.61 um	yes	10 mm	76

4. CONCLUSIONS

a) The Gamma rays are ionizing radiation, therefore can kill all the bacteria. The killing of bacteria accomplished by destroying the cell's chromosomes with radiation. Radiation ionizes the atoms and molecules creating charged particles and free radicals. Free radicals can cause breaks in the DNA structure.

b) The RF sputtering techniques help to improve ceramic structure in form of coated add to convert the particle from micro to nano size, Al_2O_3 and TiO_2ceramic have natural antibacterial materials, with nano size possess greater antibacterial properties as particle size is reduced into the nanometer regime (force of surface increasing due to the increased surface to volume ratio of a given mass of particles), the physical structure of a nanoparticle itself and the way in which it interacts with and penetrates into bacteria appears to also provide unique bactericidal mechanisms.

c) The inhibition of bacteria was increased with increasing thickness of thin film. The increasing thickness of film the increasing absorption A and radiation R of film for dose radiation gamma ray lead to increasing percentage inhibiter bacteria growth.

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