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RESEARCH PAPER

The Effect of TiO_2, TiN, TiO_2/TiN Thin Film Coatings on the Antibacterial Properties of Ti6Al4V Biomedical Alloy

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ABSTRACT

Titanium (Ti) implants are materials that are widely used in dentistry and orthopedics. They have important properties such as corrosion resistance, biocompatibility, and mechanical properties, which are suitable for these applications and have given them increasing interest in medical and practical applications. In this research, the sputtered thin films coated medical titanium alloy (Ti6Al4V) is an enhancement for the replacement of biological bones and teeth to become more corrosion-resistant and enhance antibacterial properties. The enhancement process was achieved by coating the alloy with materials with good medical application properties. Using a DC reactive sputtering with (300) watts of power sputtered to deposit one layer of TiO_2, one layer of TiN, and multilayer film (TiO_2/TiN) on Ti6Al4V alloy substrates. The structural properties, surface morphology, and antibacterial tests of the prepared samples were studied. The particles' structural characteristics, arrangement, and distribution were obtained by scanning electron microscopy and atomic force microscope examinations. The results demonstrated particles of coating layers deposited at a sputtering power of 300 watts having a uniform, homogenous distribution. The results obtained by examining the antibacterial properties of (Ti6Al4V) samples before and after deposition with different types of thin films showed that the highest value of antibacterial properties was multilayer (TiN\TiO_2) on Ti6Al4V alloy substrates.

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INTRODUCTION

Materials are utilized in biomedical applications in a variety of forms, and they each need a certain combination of properties to function. Metal implants are often used, and to work within the bodies of people and animals, these materials must possess specific properties [1-4]. The researchers were interested in developing the mechanical and biological properties of the medical alloy Ti6Al4V so that it would be highly effective when used in the human body [5-15]. The biomechanical characteristics of biomaterials,

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as they are also called, are anticipated to be comparable to those of autogenous tissues without adverse effects. Biocompatibility, bio adhesion, bio functionality, corrosion resistance, among the qualities that determine whether a material is suitable for biomedical implant applications are and antibacterial activity [16]. Implants and other biomedical devices are subject to regulation by a variety of organizations across the world, including the International Standards Organization (ISO) and the U.S. Food and Drug Administration (FDA), to assure their safety and to achieve the intended objectives. The main metallic biomaterials include stainless steels, cobalt alloys, titanium, and titanium alloys [17]. Antibacterial activity is related to substances that kill or retard the growth of bacteria locally while not generally being hazardous to nearby tissue. Most modern antibiotics are either pure natural products or natural compounds that have undergone chemical modification. Nanoparticles (NPs) have gained significant uses in the domains of science and engineering in recent years and are a possible replacement for traditional materials. The enhanced surface-to-volume ratio and the greater number of atoms at NPs' grain boundaries are what give them their singularity. The development of numerous innovative devices utilized in various biological, physical, pharmacological, and biomedical applications has proven them to be important materials. [18-20]. The objective of this work is to investigate the effect of TiO_2, TiN, and TiO_2/TiN multi-layer coatings deposited on a Ti6Al4V alloy substrate via the reactive DC sputtering process and to analyze its resultant surface and anti-bacterial properties

The physicochemical characteristics of NPs and the type of bacterium are two important aspects that influence the antibacterial activity of NPs. NPs must come into touch with bacterial cells to function as antibacterial agents. Receptor-ligand interactions, van der Waals forces, electrostatic attraction, and hydrophobic interactions are all recognized contact modalities. DNA, lysosomes, ribosomes, and enzymes are all essential components of bacterial cells, and when NPs interact with them, oxidative stress, heterogeneous changes, cell membrane permeability changes, electrolyte equilibrium issues, enzyme inhibition, protein deactivation, and altered gene expression follow. Oxidative stress, metal ion release, and non-oxidative processes have all been often highlighted in recent investigations [21]. The biocidal and biostatic effects of metal oxide nanoparticles are notably ineffective against both Gram-positive and Gram-negative bacteria [22]. Fig. 1 outlines the various pathways associated with the antibacterial activities of metal nanoparticles.

MATERIALS AND METHODS

On Ti6Al4V substrates, thin films of TiO_2, TiN, and TiO_2/TiN multi-layers were deposited using a DC magnetron reactive sputtering technique. Highly pure titanium Ti (99.99%) as the target disc that was 2 inches in diameter and 3 millimeters thick and medical Ti6Al4V alloy plates as the substrate for depositing TiO_2, and TiN layers. An electric field is created for the gas discharge by the cathode that is facing the anode. The electrode poles are spaced 5 cm apart. This discharge occurs

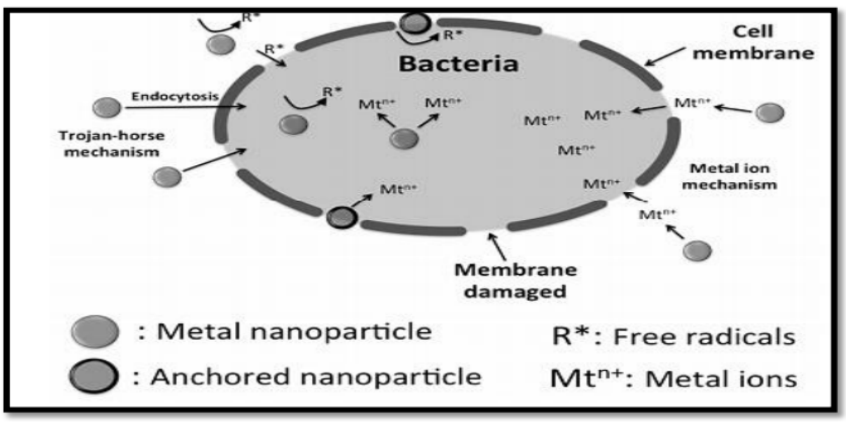

Fig. 1. An overview of the processes involved in metal nanoparticles' antibacterial activity [23].

Table 1. Reactive DC sputtering conditions for depositing TiO_2, TiN, and TiO_2/TiN thin films

Operating parameters	Values
Working pressure (Torr)	$4x10^{-2}$
Sputtering power (Watt)	175,200,250,300
The target to substrate distance (cm)	5
Substrates	Glass and Ti6Al4V
Gases mixture ratio (Ar: O2), (Ar: N)	(90:10)
Deposition time (hour)	1
Substrate temperature	373ºK

using a mixture of argon and oxygen gas (Ar: O_2) in a (9:1) ratio to coat with TiO_2 thin film and using a mixture of argon and nitrogen gas (Ar: N) in a (9:1) ratio to coat with TiN. The working pressure was equal to $4x10^{-2}$ mbar and the sputtering power was equal to (300) watts. The DC sputtering conditions for depositing TiO_2, TiN, and TiO_2/TiN thin film as shown in the following Table 1.

The antibacterial potential of the created samples (Control, TiO_2, TiN, and TiO_2/TiN) was assessed using an agar well diffusion assay against Gram's negative and Gram's positive bacterial

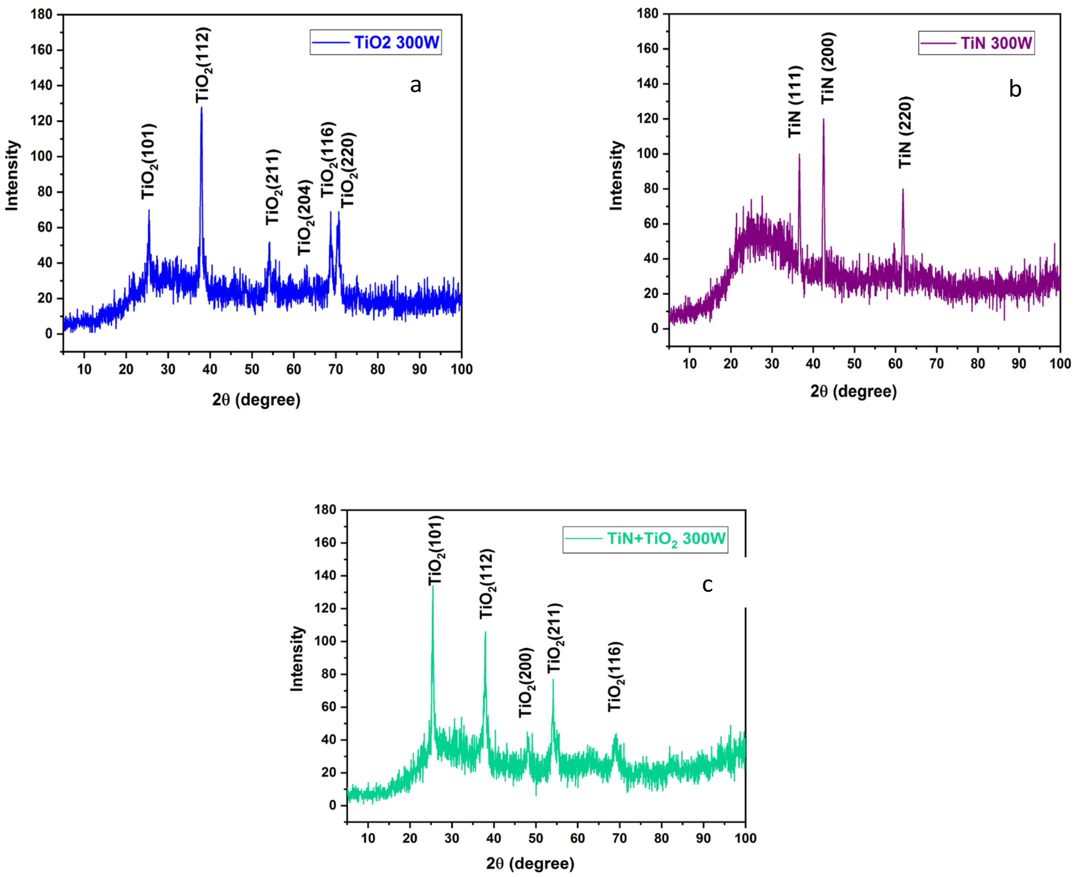

Fig. 2. XRD pattern for (a) TiO_2, (b) TiN and (c) TiO_2/TiN coatings.

strains (E. faecalis and E. coli). Amounts of around 20 ml of Muller-Hinton (MH) agar were aseptically placed in sterile Petri plates [24, 25]. The bacteria were removed from their stock cultures using a sterile wire loop [26]. After the organisms had been cultivated, 6 mm-diameter wells were drilled into the agar plates using a sterile tip. The samples (Control, TiO_2, TiN, and TiO_2/TiN) were injected into the bored wells at various concentrations. The cultured plates containing the

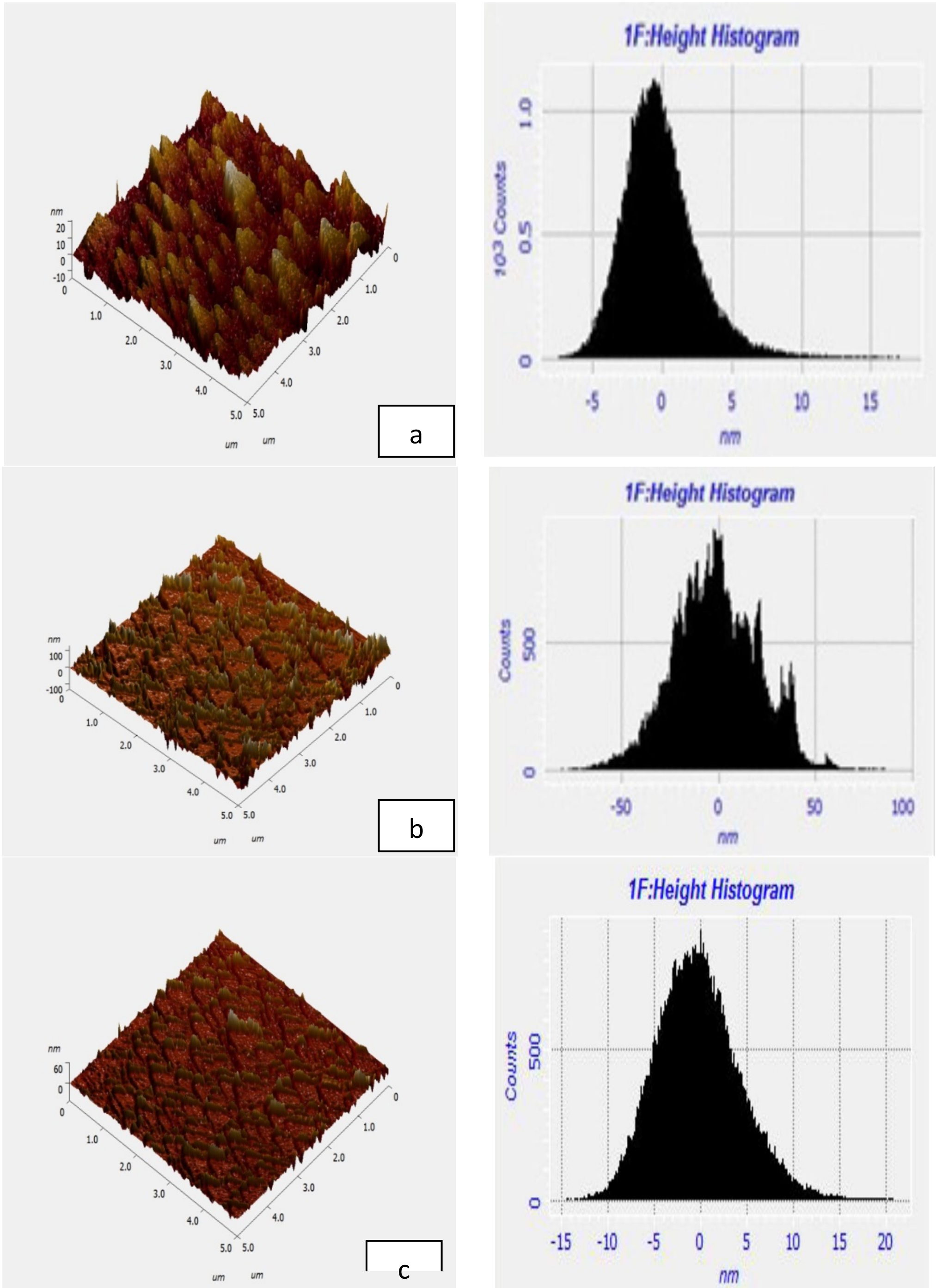

Fig. 3. 3D AFM image and histogram of of (a) TiO_2, (b)TiN and (c)TiO_2/TiN thin films.

Table 2. Average diameter, Average roughness, and the root mean square for TiO_2, TiN, and TiO_2 / TiN coatings

Sample	Sputtering Power (Watt)	Thickness (nm)	Average diameter (nm)	Average roughness (nm)	Root mean square (nm)
TiO_2	300	167	4	2.024	2.662
TiN	300	180	25	17.487	21.876
TiO_2 / TiN	300	255	5	3.525	4.468

Samples (Control, TiO_2, TiN, and TiO_2/TiN) and the test organisms were incubated overnight at 37°C before measuring and recording the average zones of inhibition diameter [27,28].

RESULTS AND DISCUSSION

The XRD patterns in Fig. 2-a show that the TiO_2 films were prepared at sputtered power (300) watts are polycrystalline with peaks locations (25.28º, 38.57º, 55.06º, 62.68º, 68.760º and 70.31º) corresponding crystal orientation (101), (112), (211), (204), (116) and (220) respectively. This pattern improves that; this film is (TiO2) Tetragonal crystals (anatas) structure. This peak is consistent with the standard data (card No. 96-720-6076). The XRD patterns in Fig. 2-b showed three peaks on the positions at 36.66°, 42.59° and 61.81 corresponding to the orientation (111), (200), and (220) respectively, referring to polycrystalline TiN cubic crystal structure. This peak is consistent with the standard data (card No. 03-065-5774). It is clear from Fig. 2-c that there are peaks belonging to the compounds (TiO_2), which means that the X-ray diffraction occurred from the first layer compounds. Part of the X-ray was reflected from the surface of the composite crystals of (TiO_2) disappears the other part was reflected from the surface of the TiN crystals. It is known that among the titanium oxides (TiO, TiO_2, Ti_2O_3. Ti_3O_5), the best hemocompatibility is exhibited by rutile with a tetragonal structure [29, 30]. It is also possible to improve the antithrombogenicity properties by modifying the phase composition of TiO_2 by producing a layer composed of a titanium nitride and titanium oxide (TiN/TiO_2) mixture [31].

AFM is ideal for quantifying the examination of the dimensional surface roughness of nanoparticles and for seeing the surface Nano-texture of the produced film coating. TiO_2, TiN, and TiO_2/TiN thin film coatings with sputtering power (300) watts thin films were studied for their surface morphology using AFM. Three-dimensional AFM pictures of samples are shown in Fig. 3a, b, and c. The homogenous distribution of the columnar structure is visible in every photograph. The TiO_2/ TiN multilayer coatings' AFM picture revealed Fig. 3c a thick microstructure.

Table 2 displays the average diameters, root mean square (RMS), and the average roughness of the films. The diameter of all the samples was found to be in the Nanoscale. From Table 2 The grain size values of the samples coated with TiO_2, TiO_2/TiN, and TiN thin films were (4, 5, and 25) nm respectively, the highest value of grain size was for samples Ti6Al4V coated with TiN because the difference between the bulk modules between TiN and Ti6Al4V is less than the difference between the values of titanium oxide and Ti6Al4V and less than the difference between the values of TiO_2/TiN multi-layer and Ti6Al4V. The results showed the decreasing values of RMS roughness and average roughness with a decrease in the values of grain size, and this is very natural, as the relationship is positive between them, as the less grain size, the lower roughness values, due to the decrease in the distance between the top and bottom of the surface topography.[32]

High magnification (60. kx) surface morphology of thin TiO_2 film, TiN, and TiO_2/TiN films are shown in Fig. 4 using a FESEM. The thin film surfaces are thoroughly coated with a consistent and homogenous distribution of particles, as evidenced by FESEM pictures. High magnification of a dark and light region of a TiO_2/TiN sheet reveals a radically distinct morphology. The dark region displays non-uniform and material aggregation film growth problems. A bright spot reveals a cluster of particles with many holes all over the surface. High-magnification SEM pictures show that the TiO_2/TiN sheet has rougher surfaces. Additionally, the range of particle sizes is (37 to 94) nm. The particle size of TiN thin film is large as a comparing with TiO_2 thin film because of the leasing of the different values of bulk modulus between the thin film and substrate. It leads to an increase in the grouping of the grains with each other, so the particle size increases. The obtained results on the FESEM microstructure of the coatings of TiO_2 and TiN compounds are consistent with the previously published studies [33, 34]

The pathophysiology of bacterial infections and implant failure is thought to include bacterial adhesion to the implant surface. The antibacterial activity was tested after coating Ti6Al4V alloy samples with films (TiO_2, TiN, and TiO_2/TiN multilayer). Gram-negative bacteria E. coli and Gram-positive bacteria E. faecalis are the two types of bacteria that TiO_2, TiN, and TiO_2/TiN film nanoparticles affect. The effects are shown in Figs. 5-8, and Table 3 summarizes the inhibition zone of nanoparticles of TiO_2, TiN, and TiO_2/TiN multilayer coatings deposited at a sputtering power of 300 watts, constant working pressure, and deposition time. According to several studies [35, 36], the thickness utilized, together with the associated grain diameter and roughness, affects the antibacterial activity. The value of suppressing bacterial growth for the smallest particle is greater. The results also showed that the highest manufactured sputtering power films had the highest value of bacterial growth inhibition. This may be explained by the increased coating layer thickness and grain roughness shown in the AFM measurements. The number of titanium ions emitted grows as the thickness is reached while utilizing high sputtering power. The interfacial

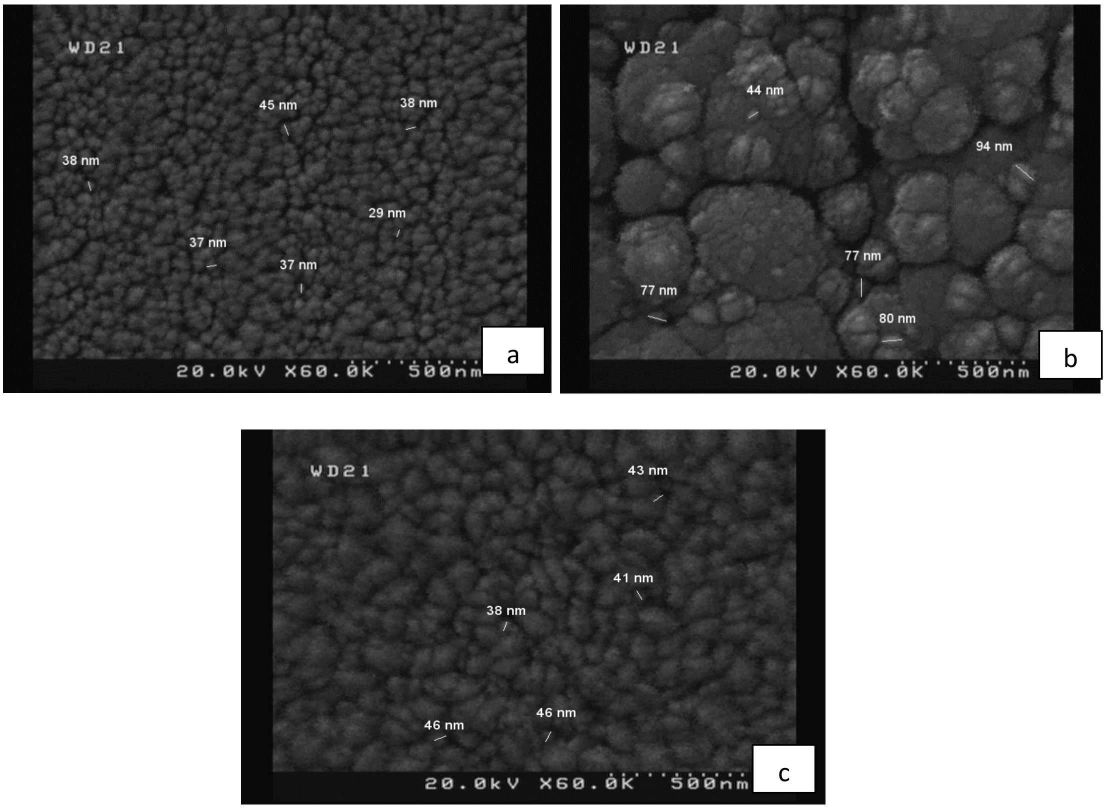

Fig. 4. FESEM image of (a) TiO_2, (b)TiN and (c)TiO_2/TiN thin films.

contact between the bacteria and the surface of the coated layer, however, increases when the roughness is increased. Therefore, as thickness and roughness increase, so does the action against germs. Furthermore, Gram-positive bacteria (such as E. faecalis) are more negatively impacted than Gram-negative bacteria (such as E. coli). This is caused by the different cell wall thicknesses. The cause may be due to bacteria's nanoparticles adhering to the bacterial cell wall and then moving inside it, which results in cell death, or it may be due to the formation of free titanium nanoparticles upon contact with the bacteria, as these free radicals can cause the cell membrane to become porous and ultimately die. Similar antibacterial effectiveness for nitrided Ti6Al4V alloy was reported by Chan C.-W et al. in 2021 [37] after it was tested in vitro for 24 hours against two of the pathogens most frequently associated with orthopedic infections, Staphylococcus aureus and

Fig. 5. Antibacterial activity against E.faecallis. (Control , 4(TiO_2)).

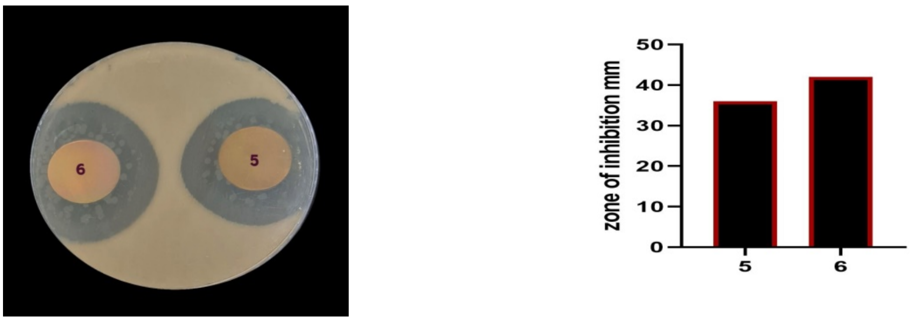

Fig 6. Antibacterial activity against E.faecallis. (5(TiN) , 6(TiO_2/TiN).

Fig. 7. Antibacterial activity against E.coli (Control, 4 (TiO_2).

Fig. 8. Antibacterial activity against E.coli 5(TiN) , 6(TiO_2/TiN).

Table 3. antibacterial activity of nanoparticles

Samples		Thickness (nm)	Zone of inhibition (mm)
E. Faecallis	Control		26
	TiO_2	169	33
	TiN	180	36
	TiO_2/TiN	255	42
E.coli	Control		24
	TiO_2	169	27
	TiN	180	32
	TiO_2/TiN	255	39

Escherichia coli.

CONCLUSION

Nanoscale TiO_2, TiN, and TiO_2/TiN multilayer coatings were successfully deposited on Ti6Al4V substrates by reactive DC sputtering magnetron sputtering. X-ray diffraction analysis reveals that the films are polycrystalline in nature with a Tetragonal crystal (anatase) structure for nanoscale TiO_2 and cubic crystal for TiN coatings. The TiO_2/TiN multilayer coated Ti6Al4V samples show better antibacterial activity than the single-layer and uncoated specimens. Thus, TiO_2, TiN, and TiO_2/TiN coatings, which have unique morphological and mechanical properties complex, can be used as an upper layer for Ti6Al4V implants and stents.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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