

Antibacterial activity and mechanism of the silver nanoparticle in gram positive and negative bacteria

Sarah K.Taha¹, Husham K. Oudah², Amal A. Halob², Mohammed K. Khalaf²,
Sabah N. Mazhir¹

¹Department of Physics, College of Sciences for Women, University of Baghdad, Baghdad,
Iraq

²Ministry of Science and Technology, Baghdad, Iraq

E-mail: hishamalshweli@yahoo.com

Abstract

Biomedical alloy 316L stainless steel enhancing to replace biological tissue or to help stabilize a biological structure, such as bone tissue, enhancing were coated with deposition a thin layer of silver nanoparticles as anti-bacterial materials by using DC- magnetron sputtering device. The morphology surface of The growth nanostructure under the influence of different working pressure were studied by atomic force microscope. The average grain size decrease but roughness of the silver thin layer was increased with increasing the working pressure. The thickness of silver thin layer was increased from 107 nm at 0.08 mbar to 126 nm at 1.1 mbar. Antimicrobial activity of silver thin layers at different working pressure were studied. The results showed that the increasing in working pressure, lead to increase in activity of silver thin coating layer against the bacteria as a result of increasing in thickness and roughness of thin coating layer. This work has been extended to study the anti-bacterial activity were found the diameters of inhibition zone of gram positive bacteria between 16.5 ± 1.5 and 19 ± 0.5 while the diameters of inhibition zone of gram positive bacteria between 17 ± 1 and 26 ± 1 . Finally the measurements of the 316L alloy coated by silver nanocoating layer after immersing the in simulated body fluid (SBF) solution for one month is the XRD pattern for the sample showed obviously that the Hydroxyapatite layer was appeared at ($2\theta = 31.8$).

Key words

Bio-metal alloy, high anti-bacterial effect, biocompatible material.

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النشاط المضاد للبكتيريا وآلية الجسيمات النانوية للفضة في البكتيريا الموجبة والسالبة لصبغة

غرام

سارة خضير طه¹، هشام كاظم عودة²، أمل عبد النبي حالوب²، محمد خماس خلف²، صباح نوري مزهر¹

¹قسم الفيزياء، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق

²وزارة العلوم والتكنولوجيا، بغداد، العراق

الخلاصة

تم في هذا العمل، تحسين سبائك الفولاذ المقاوم للصدأ الطبية نوع L 316 لتحل محل الانسجة البيولوجية أو المساعدة في تثبيت البنية البيولوجية، مثل الانسجة العظمية للمساعدة في عملية الاستشفاء. وقد تمت عمليات التدعيم عن طريق طلاء بمادة الفضة باستخدام منظومة التريز الماكنتروني ذات التيارات المستمرة. درست طبوغرافية السطح للتركيب النانوي المتكونة تحت تأثير ضغوط تشغيلية مختلفة. نلاحظ ان قطر الحبيبة يقل مع زيادة الضغط، في حين ان معدل الخشونة يزداد مع زيادة الضغط المستخدم. بالإضافة الى ذلك نلاحظ زيادة سمك طبقة الطلاء من 107 nm عند ضغط 0.08 mbar الى 126 nm عند ضغط 1.1 mbar. كما درست الفعالية المضادة لنوعين من البكتريا لطبقات طلاء الفضة المحضرة في ضغوط تشغيلية مختلفة. وأظهرت النتائج ان الزيادة في ضغط التشغيل يؤدي إلى زيادة في الفعالية ضد البكتيريا نتيجة لزيادة في سمك و خشونة طبقة طلاء الفضة الرقيقه. حيث تراوحت اقطار التثبيت بين 16.5 ± 1.5 و 19 ± 0.5 للبكتريا الموجبة لصبغة غرام في حين تراوحت اقطار التثبيت بين 17 ± 1 و 26 ± 1 للبكتريا السلبية لصبغة غرام. وأخيراً، قياسات سبائك L316 المغلفة بطبقة من الفضة النانوية بعد غمرها في محلول مذيب (SBF) في الجسم لمدة شهر واحد هو نموذج XRD للعينة أظهر بوضوح أن طبقة الهيدروكسيباتيت عند الزاوية ($\theta = 31.8$).

Introduction

A biomaterial is a nonviable material used in a medical device, intended to interact with biological systems, Any material of natural or of synthetic origin that intended to interface with tissue, blood or biological fluids, and intended for use in prosthetic, diagnostic, therapeutic or storage application without adversely affecting the living organism and its components. Polymeric biomaterials, Bioceramics, Metallic biomaterials, Biocomposite and Biologically based (derived) biomaterials. [1, 2] Biomaterials are used to make devices to replace a part or a function of the body in safe, reliably economically, and physiologically acceptable manner.

Thus, biomaterial helps in improving the quality of life and longevity of human beings and the field of biomaterials has shown rapid growth to keep with the demands of an aging population. Biomaterials are used in different parts of the human body as artificial valves in the heart, stents in blood vessels, replacement implants in shoulders, knees, hips, elbows, ears and orthodontic structures. A variety of devices and materials are used in the treatment of disease or injury. Commonplace examples include suture needles,

plates, teeth fillings, etc. [3, 4] Metals and its alloys widely used as biomaterial because its Strong material Ductile, Relatively easily formed into complex shape High modulus and yield point, make them suitable for bearing large load without leading to a large deformations and permanent dimensional change [5, 6].

Metallic biomaterials continue to be used extensively for the fabrication of surgical implants primarily for the same reason that led to their initial selection for these devices many decades ago. The high strength and resistance to fracture that this class of material can provide, assuming proper processing, gives reliable long-term implant performance in major load-bearing situations. Coupled with a relative ease of fabrication of both simple and complex shapes using well-established and widely available fabrication techniques (e.g., casting, forging, machining), this has promoted metal use in the fields of orthopedics and dentistry primarily, the two areas in which highly loaded devices are most common although similar reasons have led to their use for forming cardiovascular devices (e.g., artificial heart valves, blood conduits and other components of heart assist devices, vascular stents), and neurovascular implants (aneurysm clips). In addition,

the good electrical conductivity of metals favors their use for neuromuscular stimulation devices, the most common example being cardiac pacemakers. These favorable properties (good fracture resistance, electrical conductivity, formability) are related to the metallic interatomic bonding that characterizes this class of material [7, 8].

Metal nanoparticles are used in insecticide and bactericide for many years. Metal nanoparticles exhibit different antibacterial properties according to the surface to volume ratio. Gram positive bacteria exhibit greater resistance in contrast to metal nanoparticles compared to gram-negative bacteria, this could be related to the structure of the cell wall [6].

"In 2014 Carvalho et al studied the influence of coatings morphology and thickness on antimicrobial performance of the product samples. The results attained have shown that the deposited thin films present columnar morphology. The increase of ZnO coatings thickness until 200 nm increases the active surface area of the columns. The thinner samples (50 and 100 nm) present a less pronounced antibacterial activity than the thickest ones (200-600nm). Regarding Ag-doped ZnO thin films, it was verified that increase. Regarding Ag-doped ZnO thin films, it was verified that increasing the silver content decreases the growth rate of *E. coli*. and decreases the amount of bacteria cells present at the end of the experiment" [9].

In 2017 Mohammad et al. was investigated the antibacterial activity of nanomaterial's copper, silver, gold and titanium dioxide separately on both gram positive and negative bacteria. Nanoparticles of Cu, Ag, Au

and TiO₂ films were grown on glass substrates by DC and RF magnetron sputtering techniques. In vitro antibacterial analysis indicated that significantly reduced number of used *Escherichia coli*, *Pseudomonas aeruginosa*, and *staphylococcus aureus* were detected on Ag nanoparticles surface compared to a coated substrate surface. Both Cu and Au nanoparticles had inhibited some of pathogenic bacteria and observed over sample area [10].

Silver nanoparticles are of interest because of the unique properties (*e.g.*, size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles [11].

Experimental part

- Plasma source

In this research, samples were prepared by using home – made magnetron sputtering as shown in Fig.1. Sputtering system consist of an evacuated chamber; cathode (a target of silver) and anode disk of stainless steel. The cathode faced the anode, which provides electric field for the gas discharge. The distance between cathode and anode is 4 cm. The target which used in sputtering process is silver. The diameter of the target is (2)cm, with presence argon gas (Ar) with flow rate (40 sccm). Different working pressure was used, where working pressure values ranging from (5×10^{-1}) to (3.5×10^{-1}) mbar. The surface morphology, films thickness and anti-bacterial activity were found.

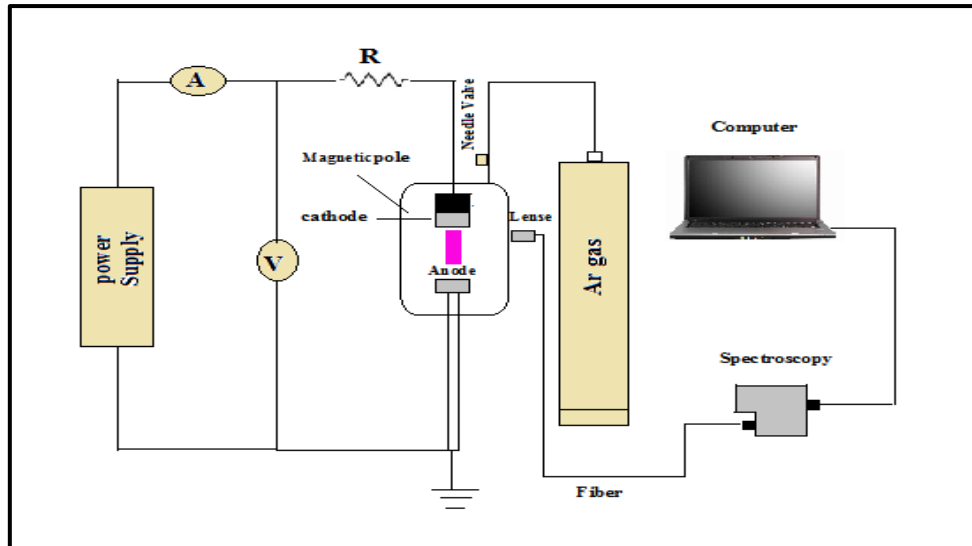


Fig.1: Experimental setup of magnetron sputtering system.

- Determination of coating layer thickness

The thickness of thin films was measured by using an optical interferometer method employing He-Ne laser ($0.632 \mu\text{m}$) wavelength with incident angle 45° . This method depends on the interference of the laser beam reflected from thin film surface and then test, the films thickness (t)

was determined using the following formula substrate.

$$t = \frac{\lambda}{2} \cdot \frac{\Delta x}{x}$$

where x is the fringe width, Δx is the distance between two fringes and λ is the wavelength of laser light. See Fig.2

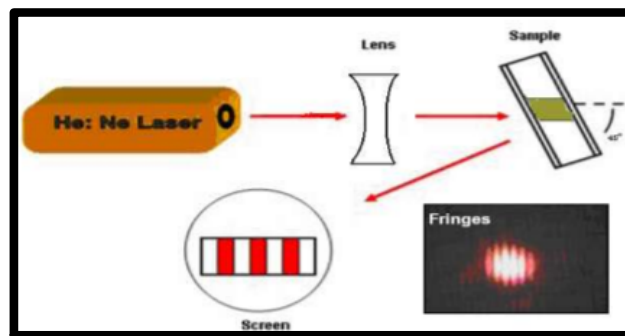


Fig. 2: The schematic diagram of the film thickness.

- Preparation of bacteria

In this project, two type of bacteria was used. The first bacteria was Gram-positive (*Staphylococcus aureus*) which isolated from pus in human skin. The second bacteria was Gram-negative (*Escherichia coli*) which isolated from urinary tract in human body. These two types of bacteria are satisfactory and cause infection. These bacteria were obtained from Ministry of Science and Technology,

Environment and Water Directorate, Baghdad, Iraq. The bacterial suspensions of the (*Staphylococcus aureus*) and (*Escherichia coli*) bacteria were prepared by specific concentrations. This concentrations was measured by ultraviolet-visible (UV-Vis) spectrophotometer device SP-3000 Plus (Optima, Japan) at 625 nm and was 0.402 nm which equivalent to a bacterial number of 6.5×10^8 cells as, in McFarland

solution. After the suspension was prepared, 0.1 ml of bacteria was spread on the 20 ml of nutrient agar via spreading method by sterile swab in standard Petri dish.

Then silver-coated samples were placed on the bacteria which prepared in the dish to study the effect of silver on the bacteria.

Results and discussion

The surface morphology of Ag films deposited on the glass substrate was studied by Atomic Force Microscope (AFM). The growth of nanostructure under the influence of different working pressure. The images have light and dark regions, where light regions represent the highest points and the dark points are the depressions. The roughness parameter and grain size are estimated by the analyzing the topography scans of the sample's surface. The surface profile parameters include average roughness (Ra) and root mean square roughness. Fig.3 shows 2D and 3D AFM images of Ag thin film deposited at different working pressure. This figure indicates that the films are uniform, and the substrate surface is well covered with grains that are nearly uniformly distributed. Also we can observe that the average size, average roughness, and the root mean square increasing with increasing the

working pressure as shown in Fig.4 a and b. This because the energy can strongly change due to change of the working pressure [12]. at an increase pressure the number of atom and molecules are increase; thus, the mean free path decreased and the collision rate of electrons with atoms is increased therefore instead of gaining energy by the electron from the electric field, more and more energy is transferred from electron to plasma species during inelastic collision, and the collision rate of electrons with atom and chamber wall is increase, thus the acquired energy of electron reduces, therefore the electron density increase [13, 14].

At pressure 8×10^{-2} we observed that the surface smother because at low pressure the electron temperature is high. At high temperature, more energy should be available for the atoms to acquire so that they may diffuse and occupy the correct site in the crystal lattice. Then average size, average roughness, and the root mean square increasing with increasing the working pressure because the electron temperature decrease. In addition to the previous reason, the increase of the crystallite size may be caused by vertical grain growth in the structure.

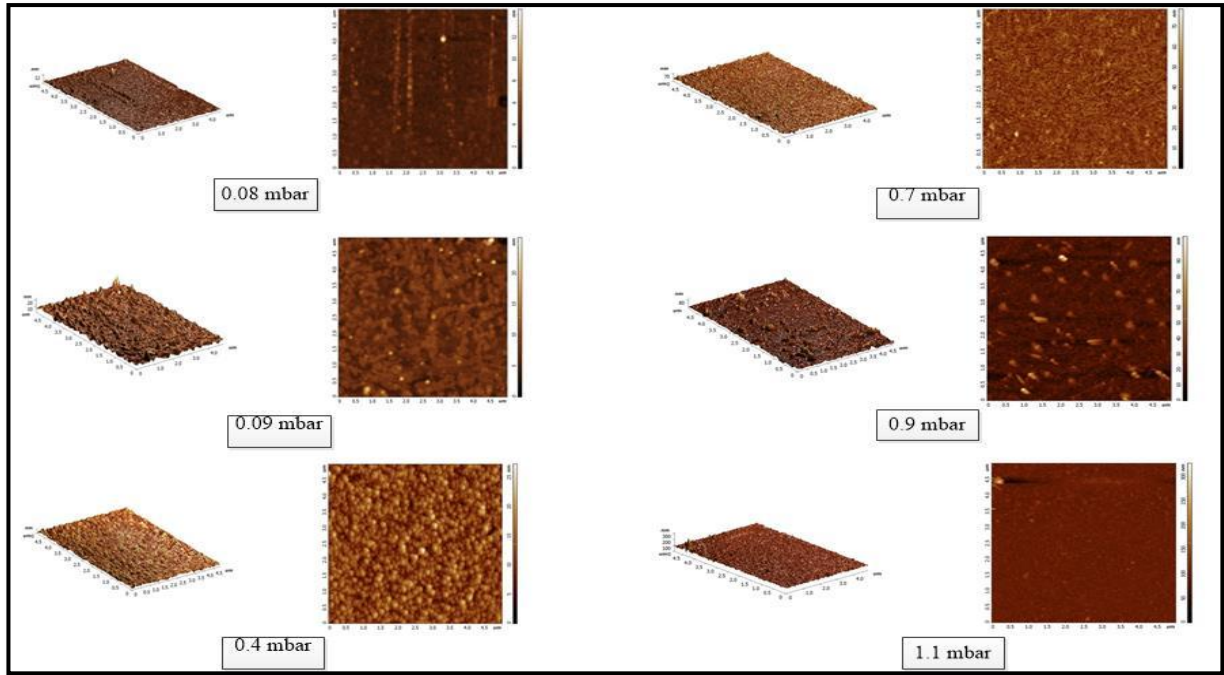


Fig. 3: 2D and 3D AFM images of Ag thin film deposited at different working pressure.

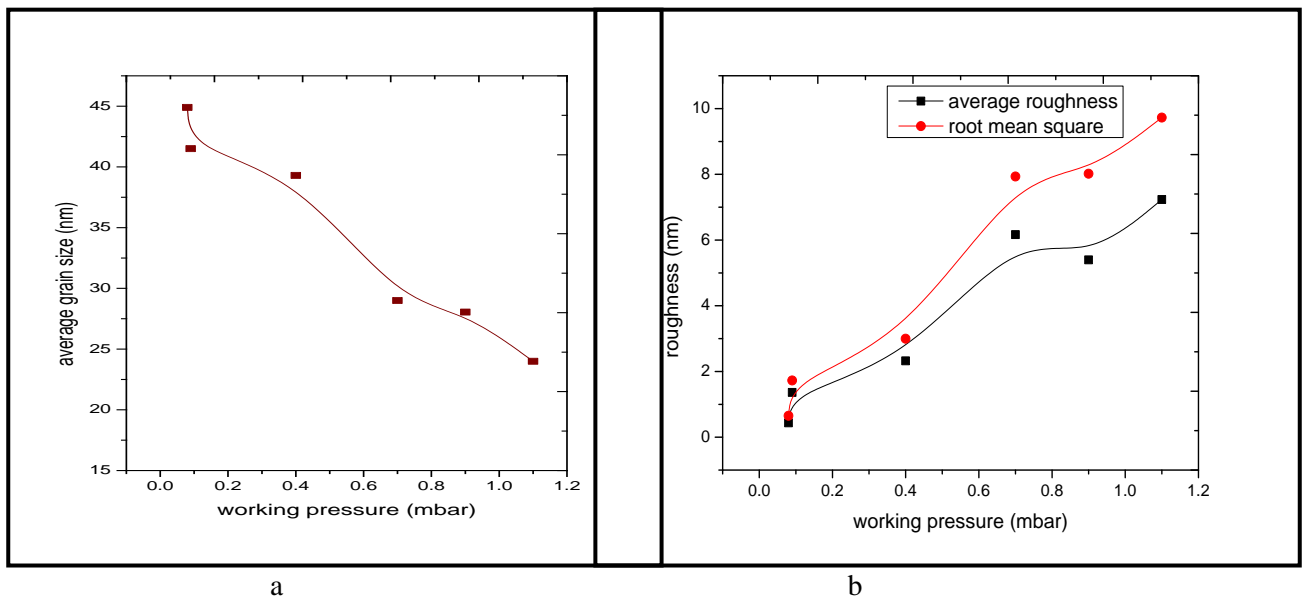


Fig. 4: Average grain size, average roughness, and the root mean square at different working pressure.

The results showed that thickness was increased as working pressure increase as shown in Table 1 and Fig.5. This because the energy can strongly change due to change of the working pressure, and we believe that the crucial parameter is the total energy delivered to the Ag films during its growth [15, 16]. At an increase

pressure the number of atom and molecules are increase; thus, the mean free path decreased and the collision rate of electrons with atoms is increased therefore instead of gaining energy by the electron from the electric field, more and more energy is transferred from electron to plasma species during inelastic collision, and

the collision rate of electrons with atom and chamber wall is increase, thus the acquired energy of electron

reduces, therefore the electron density increase [12, 14].

Table 1: The Thickness of Ag thin films deposited at different working pressure.

Working pressure (mbar)	Thickness (nm)
0.08	107
0.09	112
0.4	123
0.7	125
0.9	113
1.1	126

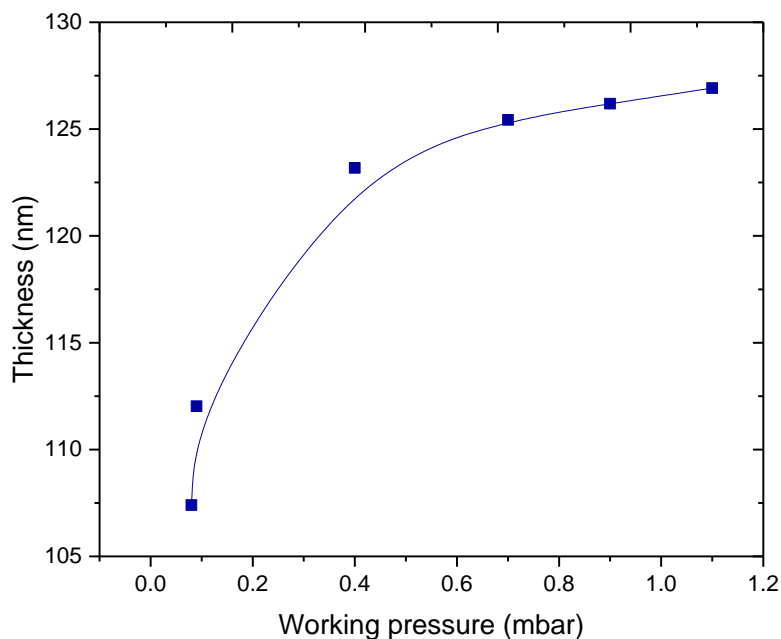


Fig. 5: Film thickness at different working pressure.

Some authors have been reporting that the smaller the particle size has the greater efficiency in inhibiting bacteria growth. Moreover, several works showed that the antimicrobial activity is also dependent on the thickness of coating layer which used.

Results show that films deposited with highest pressure has the highest antimicrobial activity this may be attributed to increase the thickness of coating layer and roughness which measure in AFM instrument. When thickness increase the amount of released Ag ions increase [15, 16]. On the other hand increasing in roughness

lead to greater interfacial contact between bacteria and surface of coating layer. So increasing in thickness and roughness lead to increase in activity against bacteria [17]. This result agrees with previous researches [15, 16]. Also we can see higher effective on Gram negative (*Escherichia coli*) than Gram positive bacteria (*staphylococcus aureus*). This due to variation of cell wall thickness, the wall of Gram-positive cells contains a thick layer (i.e., 20–50 nm) of peptidoglycan (PG), which is attached to teichoic acids that are unique to the Gram-

positive cell wall [18]. The action of antimicrobial activity is not clearly known in previous researches, the reason may be because the Silver nanoparticles have stick on bacterial cell wall and then enter it causing death of cell, or by forming free silver nanoparticles radicals when contact with bacteria, these free radicals have the ability to make porous and then damage the cell membrane. The effect for Ag nanoparticles deposited at different work pressure on the two different bacteria types (*Escherichia coli* and *staphylococcus aureus*) are depict in Fig. 6. A and B At applied voltage constant applied voltage for 30 min. Table 2 summarizes the inhibition zone effect for Ag-nanoparticle deposited at different working pressure.

Also an important result we can observe, gram-negative bacteria more affected by nano-coating more than gram-positive bacteria. According to the most common method, metal oxides NPs carry a positive charge while the microorganisms carry

negative charges (sulfur); this causes electrostatic attraction between microorganisms and metal oxides NPs leading to microorganism's oxidization and finally death. gram-negative bacteria have high containing of protein type. The most important proteins is porins through which molecules can diffuse. Unlike other membrane transport proteins, porins are large enough to allow passive diffusion. Porins will allow the materials to diffuse from surroundings of bacterial cell to inside bacterial cell when the electrostatic interaction occur. Because of porins are present in the outer membrane of gram-negative bacteria, so a large component of metallic nanoparticles inter the bacterial cell and then interact with Phosphorous (P) which is a component of nucleic acids like DNA in bacteria and finally death. So that gram-negative bacteria affected by nano coating more than gram-positive bacteria. This is consistent with previous studies [19, 20].

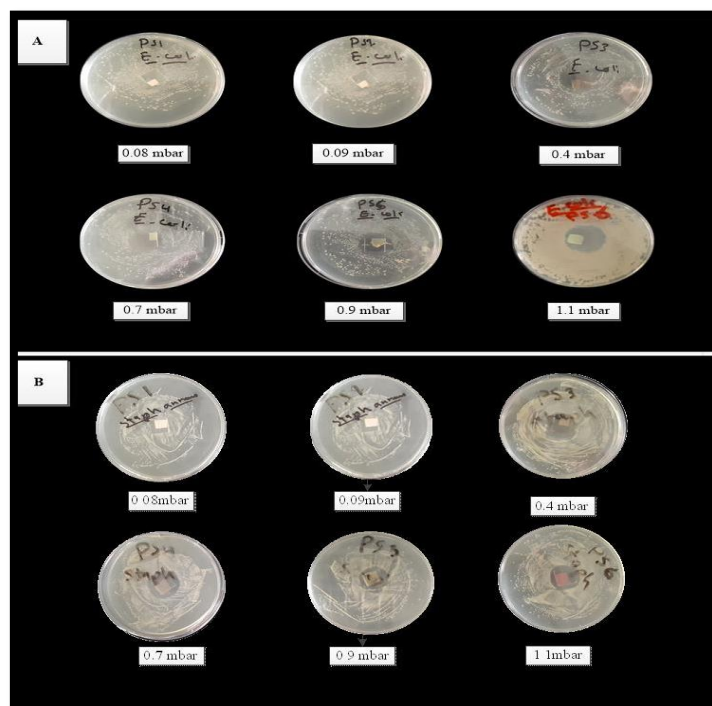


Fig. 6: Antimicrobial activity for Ag nanoparticles deposited at different work pressures on A: *Escherichia coli* B- *staphylococcus aureus*.

Table 2: The inhibition zone effect for Ag-nanoparticle deposited at different working pressure.

Working pressure(mbar)	Thickness (nm)	Average roughness	Root mean square	Inhibition zone (Staph.aureus)	Inhibition zone (E-coli)
0.08	107.4	0.437	0.652	No growth bacteria over chips	No growth bacteria over chips
0.09	112.03	1.363	1.725	No growth bacteria over chips	No growth bacteria over chips
0.4	123.18	2.324	2.997	16.5±1.5	17±1.0
0.7	125.43	6.163	7.934	17±0.0	20.5±0.5
0.9	113.19	5.396	8.017	18±2.0	22.5±0.5
1.1	126.92	7.233	9.726	19±0.5	26±1.0

The optical camera images for st.st. Alloy, Ag /st.st sample obviously show the hydroxyapatite layer formed after immersing in simulated body fluid (SBF) solution for one month.

Fig.7 shows the optical microscope images for the sample immersed in

SBF. A of hydroxyapatite layer grown on the surfaces of the sample. This layer was formed biocompatibility from simulated body fluid that has ensured the biocompatibility of the coating layer [21].

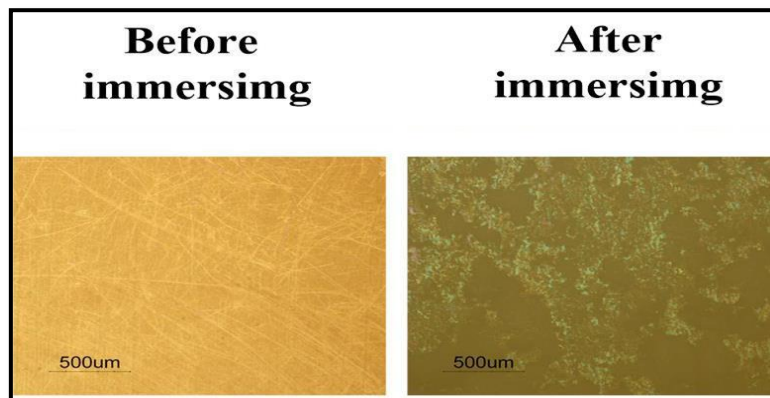


Fig. 7: The optical microscope images for the sample immersed in SBF.

We observed a gel layer formed in bottom of the container where the biomimetic treating was occurred, and after filtration and drying for this formed gel layer and sintering at 400 C°, the XRD pattern shows that a hydroxyapatite layer was formed.

Ag coating on stainless steel act as a connection between stainless steel and bones in human body. A hydroxyapatite layer formed in the

solution and then deposit on the coating layer. We observed that the hydroxyapatite layer, which formed on silver coating, has high thickness. This may be depend on stability of material coating.

The XRD pattern for the sample shows obviously Hydroxyapatite layer was appeared at ($2\theta = 31.8$). See Fig.8.

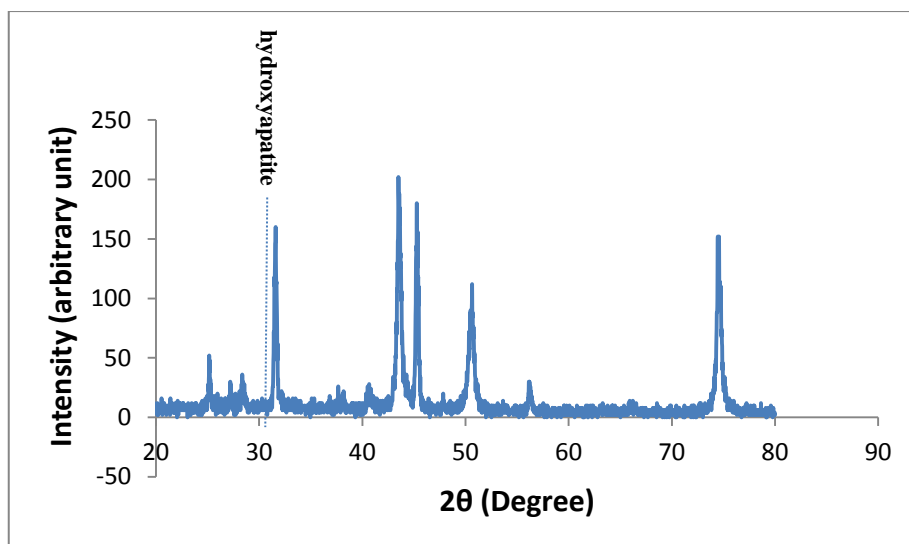


Fig. 8: The XRD pattern for the sample, which show a hydroxyapatite peak.

Conclusions

In conclusion, it is evident that this study has shown the effect of changing working pressure on surface morphology and bacteria. The results were shown that the increase in working pressure could be contributed to increase films activity on bacteria. The sputtered nanostructures thin films, average roughness and root mean square were increased with increasing working pressure. Also the results indicate that the average grain size decrease with increasing working pressure. Also an important result we can observe, gram-negative bacteria more affected by nano-coating more than gram-positive bacteria. Biocompatibility test shows the hydroxyapatite layer was grown on the surface of coated sample and this refer to the biocompatibility of prepared sample.

From all results above, we can conclude that the sample can be used as bone substitutes. Because the prepared sample act as anti-bacterial and it is biocompatible material.

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