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Study the antibacterial activity of hydroxyapatite- nano silver coating on titanium substrate

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Abstract. The most often encountered laborer as a consequence prosthetic titanium (Ti) implant, failure is infection. Implant surfaces that are antibacterial due to nanoscale titanium modifications appear as an appealing peri-implantitis prevention approach. In this study, composite coating preparation) and antimicrobial properties (hydroxyapatite, hydroxyapatite/nanosilver) on titanium substrate by Micro Arc Oxidation(MAO). Preparation the electrolyte in the electrolyte, cell was an aqueous calcium acetate monohydrate solution of 0.1.3 mol/L ((CH₃-COO)₂.Ca.H₂O) and 0.0.6 mol/l sodium biphosphate dihydrate (NaH₂PO₄.2H₂O) in distilled ,water, pH of electrolyte was 2.3 after that, nano silver was added to the electrolyte in different proportions (0.5, 1, 1.5, 2) g/L, and the coating times were also different (30, 45, 60) sec at a constant voltage 200 V. To observe the morphology a AFM test was done and must be known if there are bacteria present or not on Ti substrate and composite coating (HA, HA/nAg) samples by the antibacterial test. In addition, the surface, roughness was measured to study the extent of bacterial adhesion. The result of the tests for coated samples better compared with the uncoated sample. As a result, in the antibacterial test, composite coatings (HA, HA/nAg) were found to be more effective than Ti samples in destroying bacteria that had formed on their surfaces.

1. Introduction:

The terms biomaterials can be defined in two options: as familiar, biological materials, tissues and woods or as any materials that take the place of living tissues or organs in their work, metallic implants are an example of biomaterials [1]. Implants are made up of various materials assortment of materials, including metals, polymers, ceramics and composites. Metals are a significant subset of these materials((Ni-Ti) alloys Mg based alloys [2], Alloys containing Co-Cr, Stainless steel and (Ti)alloys) are the presently metals used in orthopedics application such as hip and knee implant [3], these materials showed excellent strength, these products, on the other hand, release toxic elements that can lead to skin diseases [2]. Among the various metallic implant materials, titanium (Ti) is



widely used in the manufacturing of bones fixation systems, orthopedic and dental implants as osteosynthesis applications. It resembles bone in terms of mechanical properties. Titanium (Ti) It forms an oxide film that is both defensive and stable This film will improve corrosion resistance, adsorb proteins, and induce bone cell differentiation [4].

Titanium(Ti) is very light with density of $4.5 \text{ (g/cm}^3\text{)}$. Pure (Ti) as an allotropic metal having hexagonal (α – phase (HCP)) below $882 \text{ }^\circ\text{C}$ and transforming to a cubic (β – phase (BCC)) greater than the temperature . The human body recognizes these materials as strange and encases it in fibrous tissues in an attempt to isolate it. Nevertheless, they don't promote and human tissues tolerate them well and have the opposite reaction. [5].

As a result, in order to enhance biomaterials' efficiency in biological systems, there is a pressing need for their surface modification. A number of different Modifying the surface of metallic materials is possible through a variety of methods including nano phase materials that improve their cellular activities. Generally, nano scale Surfaces have a lot of surface energy which is the initial protein adsorption and it is crucial in regulating the cellular interactions on an implant surface. Surface characteristics are also significant an influence on adhesion, together with charge distribution and the chemistry of the material. The roughness of titanium (Ti) nano structures solely impact osteoblast cell adhesion, proliferation, and spreading [6]. Surface modification is known as the process of coating or altering the surface of a material using biological, physical, or chemical techniques to create materials with different functions than the original material [7].

Among the various coating techniques, micro arc oxidation MAO, a high-voltage-plasma-assisted anodic oxidation process, is a simple way to fabricate thick, porous, hard and insulating ceramic coatings [8].

MAO-treated ceramic coatings have high micro-hardness outstanding union to the alloy background, unveil abrasion resistance improved wear and corrosion struggle [9]. In the meantime the process of MAO is relatively convenient without any environmental pollution. However, the influence factors of MAO operation such as the electrolyte composition [10,11], electrochemical parameters and the type of power supply [12] have a significant influence on the overall properties of MAO coatings [9]. Benefits of this MAO operation "is the ability to incorporate (Ca)and (P)ions hooked on the surface layer by varying the composition and concentration of the electrolyte". The incorporated Ca and P ions were flush crystallized into hydroxyapatite HA or other calcium phosphates by a hydrothermal treatment [13].

Hydroxyapatite is the best example of a ceramic coatings and $\text{HA}(\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2)$ it has inorganic components that are identical to those found in bone tissue, and it has a high value for bone-substitute biomaterials. Likewise, HA coating for metal implants has been show to effectively induce chemical osseointegration in the implant bone interface [14].

Regardless of the circumstances good osteo theorem performance of (CaP)coatings, a successful implant must overcome any more challenge bacterial infection. The standard procedure vancomycin and gentamicin are examples of antibiotics. are used to fight infection. However, the hazard of antibiotic resistance is briskly become a major concern [15]. The most effective strategy is antibacterial properties are added to ceramics and materials to prevent contamination. Silver is a very appealing alternative among various inorganic antimicrobial agents because it has many advantages such good antibacterial strength, excellent biocompatibility, and satisfactory stability. $[\text{Ag}^+]$ ions are able to penetrate the bacterial cell wall and destroy the cell equilibrium [16]. $[\text{Ag}^+]$ ions can bind to nucleic acids, enzymes and membranes and thus obstruct the electron, transport chain of microorganisms[17].

In 2018, Teker Aydogan et al. studied Micro-arc oxidation(MAO) was used to increase the silver Ag content of a coating synthesised on commercially pure titanium (Cp–Ti, Grade4) for biomedical applications. The MAO reaction was carried out in electrolytes containing silver acetate ($\text{AgC}_2\text{H}_3\text{O}_2$) at concentrations ranging from 0 to 0.002 mol/l. Coatings synthesised in 0.001 mol/l AgC_2 compare favorably to the base electrolyte [18].

In 2018, Du, Q., Wei et al. studied at various applied voltages, "Micro Arc Oxidation(MAO) coatings containing countless concentration of Ca, Zn, and P elements are successfully produced on the titanium (Ti) substrate. The MAO coating has a structure with porous surfaces and is made up of anatase and rutile (TiO_2) phases after MAO treatment. In the meantime micropore size and density on(MAO)coatings have been measured altered by changing the, voltages that are applied Furthermore, the quality of integrated elements such as Zn, Ca, and P in MAO coatings has been optimized. For E. coli and S. aureus, the(MAO) coating containing Ca, Zn, and P elements has excellent antibacterial potential. As a result, combining Ca, Zn, and P elements proved to be an effective way to improve antibacterial activity [19].

In 2020, Dalavi et al. studied as a bone graft replacement, "microspheres(COS-Ag-Alg-HA) encompassing chitoooligosaccharide (COS)" coated silver nano particles "(Ag NPs) with alginate (Alg)and hydroxyapatite. Microbes that cause disease and osteoblast-like cells were used to test the formed microspheres' antimicrobial activity and biocompatibility. The findings indicate that microspheres are rigid, with heavy chemical interactions. was discovered in the materials. The microspheres produced are osteoblast-like cells biocompatible and show against Staphylococcus aureus, significant microbial inhibition. The formed microsphere is recommended as a possible candidate for bone tissue" based on the aforementioned and finding findings repair and, renewal [12]

2. Experimental Part:

2.1. Micro Arc oxidation(MAO)

Micro arc oxidation is a form of oxidation that uses a small amount MAO is a new environmentally friendly coating technique that allows for one-step shaping of ceramic coatings on aluminum, magnesium, titanium, and alloys [9]. (MAO)operation is agreed out on voltages greater than the oxygen's breakdown voltage(O) gas stratum shrouding the anode and the anode surface is defined by assured luminous electric arcs [20] as shown in figures 1 and 2. A titanium (Ti) plate with chunkiness of (0.1 mm) was cut into (10mm,10mm). The Purity of titanium plate is 99.7%. Samples before coating were ultrasonically cleaned in ethanol alcohol for 10 min using ultrasonic cleaning device. To prepare composite coating (HA, HA/nAg) on titanium substrate we used the following materials: -

1- HA electrolyte, Preparation the electrolyte in the electrolyte cell was an aqueous solution containing 1{(0.06 mol/L) sodium biphosphate dihydrate $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and (0.13 mol/L) calcium acetate monohydrate $((\text{CH}_3\text{-COO})_2 \cdot 8\text{Ca} \cdot \text{H}_2\text{O})$ in distilled water, PH of electrolyte was 2.3.

2- nano silver has been added to hydroxyapatite electrolyte in proportions (0.5, 1, 1.5 and 2) g/L.

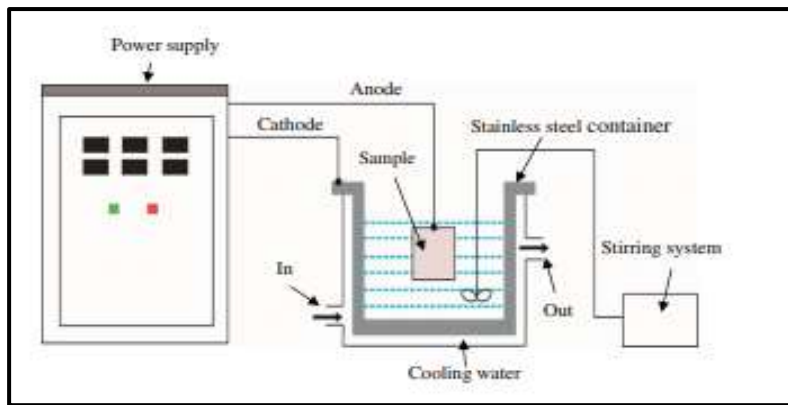


Figure 1. Schematic representation of micro-arc-oxidation setup [21].

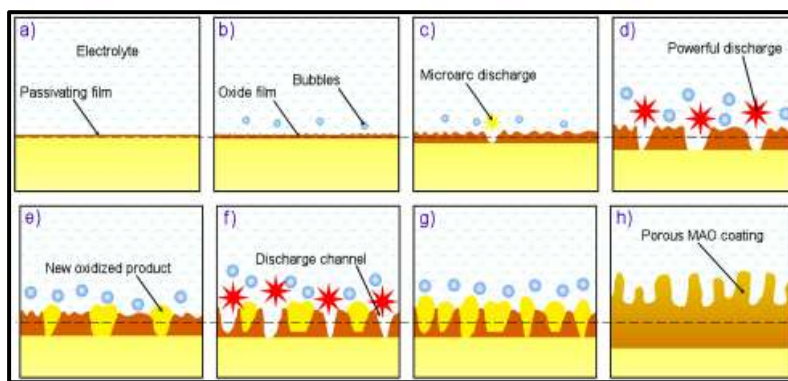


Figure 2. Schematic the (MAO) porous coating forming mechanism Initially, a passivating flick(a)and a porous insulating oxide film (b) are formed the new spawned oxide coating(e) is then formed and thickened beneath the action of spark discharges(c) and powerful arc discharges(d). Finally, there's the leaky ceramic oxide coating is twisted(h)with the endless formation and breakdown of the oxide coating(g)at thelarge discharge channels(f) [22]

There are many parameters to complete the composite coating (HA, HA/nAg) on a plate titanium, include constant voltage 200 V, time (30, 45, 60) sec and change the percentage of nano silver 0.5 g/1 L as show table 1.

Table 1. show parameters of MAO process

NO (g/L)	Sample	time	Proportion nAg
1	HA	30	-----
2	HA	45	-----
3	HA	60	-----
4	HA/ nAg	30	0.5 g/L
5	HA/ nAg	45	0.5 g/L

6	HA/ nAg	60	0.5 g/L
7	HA/ nAg	30	1 g/L
8	HA/ nAg	45	1 g/L
9	HA/ nAg	60	1 g/L
10	HA/ nAg	30	1.5 g/L
11	HA/ nAg	45	1.5 g/L
12	HA/ nAg	60	1.5 g/L
13	HA/ nAg	30	2 g/L
14	HA/ nAg	60	2 g/L

2.3 Tests:

In this work, to evaluate the performance of composite coating (HA, HA/nAg) layers on samples, the following tests were performed:

2.3.1. Atomic force microscopy test

This test used to see and measure surface structure with unprecedented resolution and accuracy. An atomic force microscope (AFM, contact mode, spm AA3000 Angstrom advanced Inc., USA) allows us, for example, to get images showing the arrangement of individual atoms in a sample, or to see the structure of individual molecules of HA/nAg composite coating.

2.3.2. Roughness test

To measure the roughness of the composite coating (HA, HA/nAg) surface on the Ti substrate, the roughness measuring device (HSR210) was utilized. This roughness on the surface can be defined as irregularity in a surface profile.

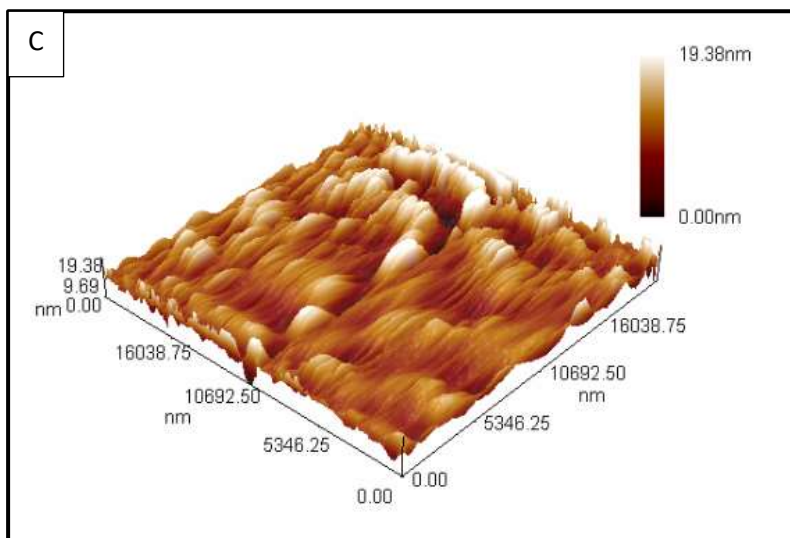
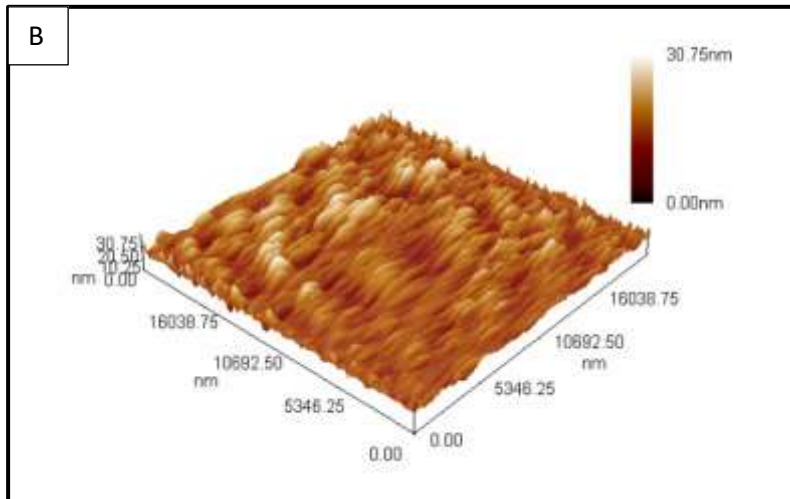
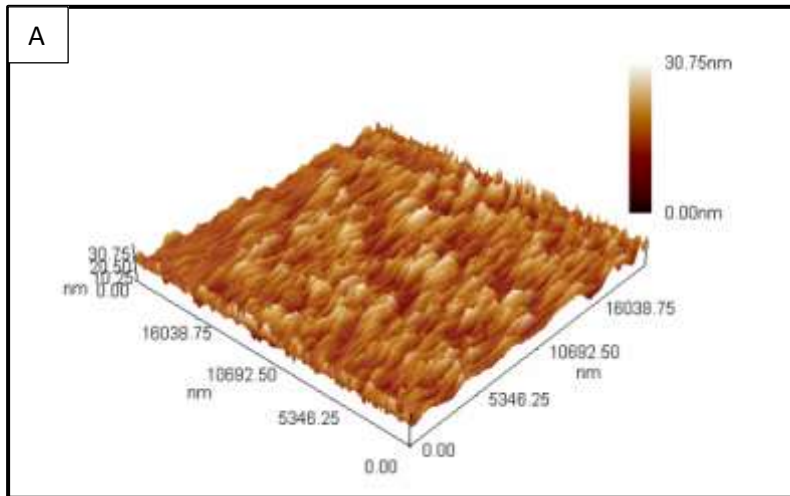
2.3.3. Antibacterial test

When biomaterials especially metallic implants are used inside the human body, they are exposed to types of bacteria that cause infections in the adjacent living cells to the metallic implant. Therefore, it must be known if there are bacteria present or not on Ti substrate and composite coating (HA, HA/nAg) samples by the antibacterial test. This test was performed by washing the sample in Petri dish with mannitol salt agar and it stayed in dish for 5 minutes. The solution was then divided into 0.5 ml and put in another petri dish to be incubated at 37 °C for 24hhours.

3. Results & Discussion:

3.1. Atomic force microscopy (AFM)

Figure (3) show the results AFM of sample for HA /nAg deposition at 60 sec. It is clear there is decrease nanoroughness of the surface by increase the proportion of silver in the electrolyte because silver is at the nano scale. The surface nanoroughness was 30.75 nm for HA & HA/0.5g/L nAg, 19.38 nm for HA/1 g/L nAg & HA/1.5g/L nAg and was 19.00 nm for HA/ 2 g/L nAg.



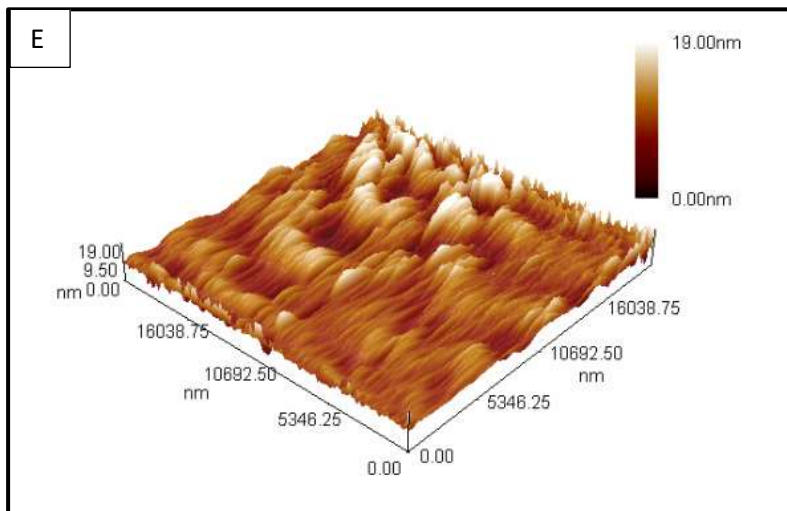
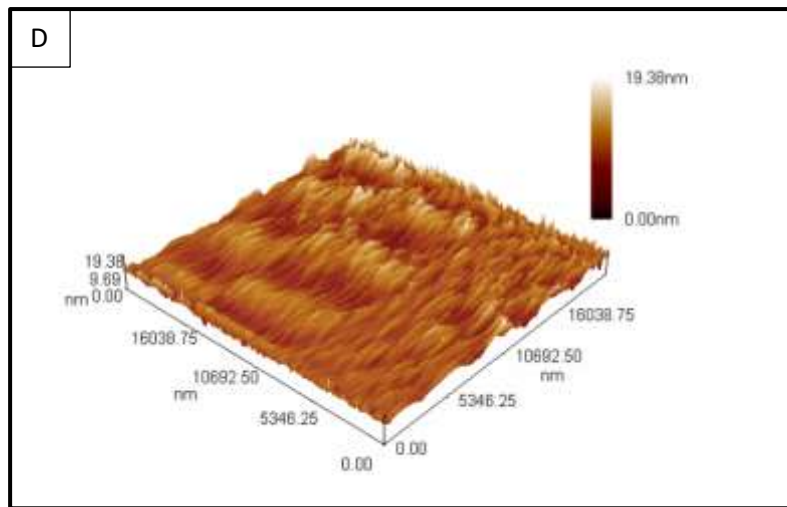


Figure 3. AFM of samples at 60 sec: (A) HA, (B) HA/ 0.5g/L n Ag, (C) HA/ 1g/ L n Ag, (D) HA/ 1.5 g/L n Ag and (E) HA/2g/L n Ag.

3.2. Roughness test:

There is difference in surface roughness between coated and uncoated (Ti) sample where surface roughness of the composite coating (HA, HA/nAg) higher than for Ti substrate because MAO method led to the cracking of the oxide to deposition the hydroxyapatite and silver ions as shown in the table (1) below. Roughness average of coated samples is (1.71 μm).

Table 2. show surface roughness of the coated and uncoated (Ti).

	Roughness (μm)		Roughness(μm)
Ti	0.038	HA/1 g/L nAg at 45 sec	1.045
HA at 30 sec	2.115	HA/1 g/L nAg at 60 sec	2.917
HA at 45 sec	1.988	HA/1.5 g/L nAg at 30 sec	0.847

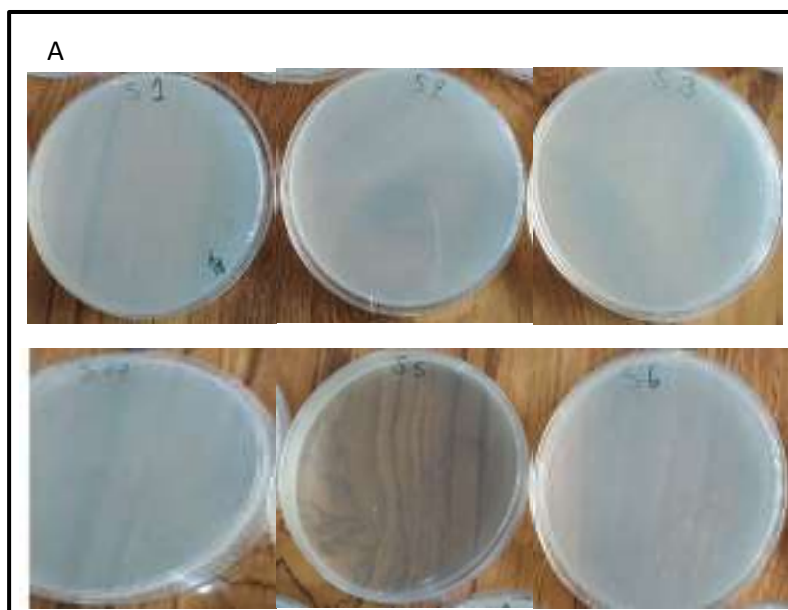
HA at 60 sec	2.625	HA/1.5 g/L nAg at 45 sec	1.283
HA/0.5g/L nAg at 30 sec 2.319	0.867	HA/1.5 g/L nAg at 60 sec	
HA/0.5 g/L nAg at 45 sec 0.883	1.472	HA/ 2 g/L nAg at 30 sec	
HA/0.5 g/L nAg at 60 sec 1.457	1.254	HA/ 2 g/L nAg at 60 sec	
HA/1 g/L nAg at 30 sec	2.473		

3.3. Antibacterial test

The effect composite coating (HA, HA/nAg) on Ti substrate was clearly effective against bacteria where this type of coating killed bacteria compared to the base sample. There was a growth of bacteria on uncoated sample (Ti) due to minimum level of roughness but no presence it on coating sample as shown in table (2) and figure (4) except for one sample (HA/1.5 g/L nAg at 45 sec) the reason for this is breakage of the coating layer within parts of a second and it caused the solution to penetrate through the cracks and reach the base metal and then rebuilds itself through MAO process.

Table 3. shown the presence of bacteria (+) or not

Sample	Result	Sample	Result
Ti (control)	+	HA/ 1 g/L nAg at 45 sec (S8)	-
HA at 30 sec (S1)	-	HA/ 1 g/L nAg at 60 sec (S9)	-
HA at 45 sec (S2)	-	HA/ 1.5 g/L nAg at 30 sec (S10)	-
HA at 60 sec (S3)	-	HA/ 1.5 g/L nAg at 45 sec (S11)	+
HA/0.5 g/L nAg at 30 sec (S4)	-	HA/ 1.5 g/L nAg at 60 sec (S12)	-
HA/0.5 g/L nAg at 45 sec (S5)	-	HA/ 2 g/L nAg at 30 sec (S13)	-
HA/0.5 g/L nAg at 60 sec (S6)	-	HA/ 2 g/L nAg at 30 sec (S14)	-
HA/ 1 g/L nAg at 30 sec (S7)	-		



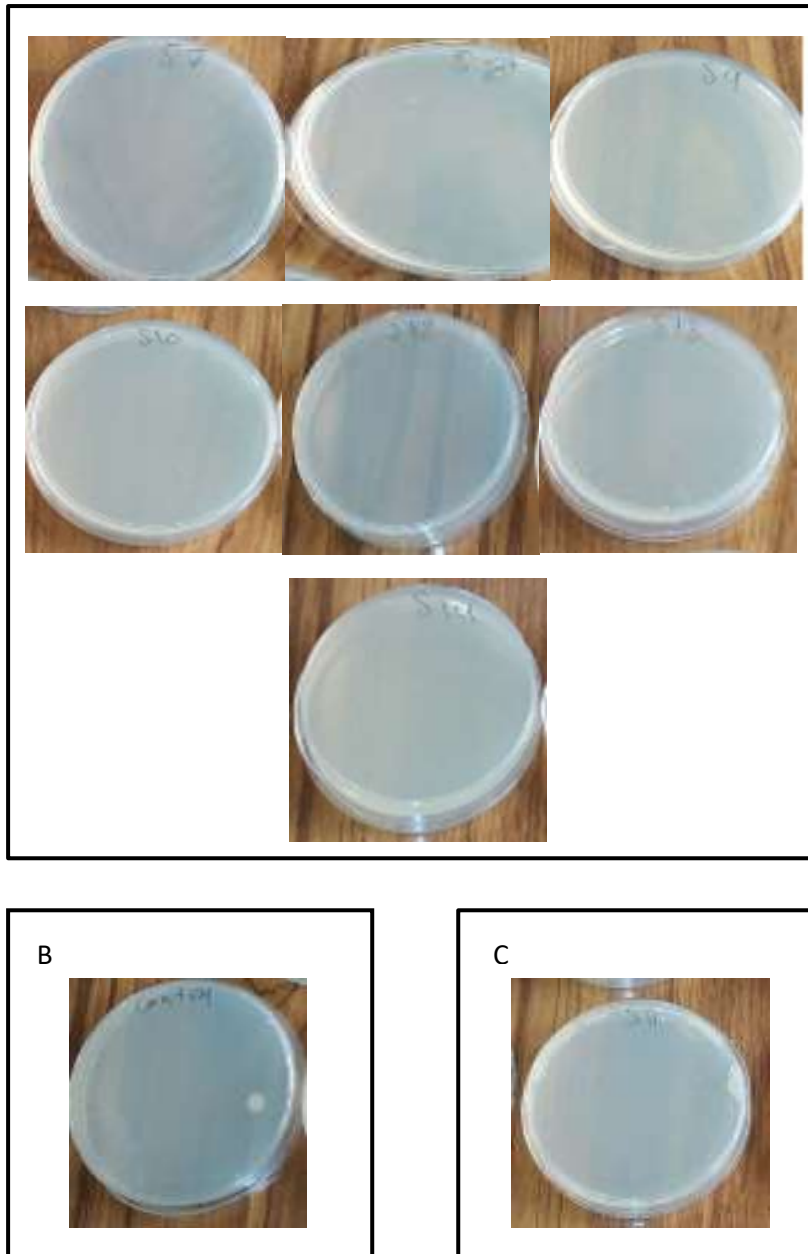


Figure 4. illustrates the comparison between (A) coating, (B)uncoating samples and (C) coating sample (HA/1.5 Ag at 45 sec (S11)).

Conclusion:

The following hypotheses are reached as a result of the findings:

1. Decrease of roughness by increase the proportion of nano silver in the electrolyte
2. Rate surface roughness for composite coating (HA, HA/nAg) is (1.71 μm) and for Ti sample is (0.083 μm), this mean that the roughness increase with coating.
3. Composite coating (HA, HA/nAg) in the antibacterial test succeeded in killing bacteria compared with Ti sample that bacteria have grown on their surface.

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