THE EFFECT OF DIFFERENT SURFACES ROUGHNESS OF Ti6Al4V ALLOY ON SILVER DOPED HYDROXYAPATITE COATING

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This paper demonstrates the effect of the Ti6Al4V alloy surface roughness on the silver characteristics doped hydroxyapatite coatings. Different sized of particles have been used to obtain different roughness of Ti6Al4V substrates by blasting process and coated by spin-coating with calcium-phosphate was produced by silver doped hydroxyapatite solution obtained by sol-gel. Scanning electron microscopy associated to energy dispersive X-Ray spectroscopy and X-ray diffraction were used to study the morphology, chemical composition and structure of the coatings. The biological characterization of the coatings was evaluated depended on the film stability in the simulated body fluid (SBF); as well as, the activity of antimicrobial and the biofilm formation by using P. aeruginosa species. The main objective of this work is to prove the influence of the Ti6Al4V surface roughness on the physico-chemical, structural and biological properties of silver doped hydroxyapatite coatings.

The most important results deduced as following:

- The layers present a good homogeneity and enhanced adhesion to the substrate.

- In turn, the surface roughness was noted that the substrate blasted with larger particles and coated with silver doped hydroxyapatite were more stable, indicating the importance of surface roughness."

Keywords: hydroxyapatite, roughness, Ti–6Al–4V alloy.

1. Introduction

The use of titanium dates back to the late 1930's [1]. Titanium alloys are desirable material for a wide range of biomedical applications because they have multidimensional properties of corrosion due to the protective thin surface oxide

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layer [2], very high biological compatibility, nontoxicity, and good mechanical properties as high strength and fracture toughness which due to stabilize the □-phase with aluminum content and vanadium which leads to the □-phase stabilizer [3,4]

On the other side, the titanium alloy has some imperfections that make it directly unusable for biomedical implants, such as lack of chemical bond between titanium alloy and bone tissue [5], existence of vanadium as toxic element which can cause noxious reactions of tissue [6] and aluminum ions as allergenic element for Alzheimer diseases [7].

Titanium alloy has low resistance for wear cause of elastic modules is higher than cortical bone by 4-6 times [8] which lead to trouble for bearing surfaces with because of ‘stress shielding’ which a rise to lead to loss and resorb of bone in addition to this alloy has low hardness value [9,10]

Nonetheless, the alloy can be presented a 'low corrosion resistance' since the oxide film is unstable, causing dissolution, in addition, generates outputs leading to the physical harm when entered to the body [10,11].

In order to overcome the surface problems arising from Ti6Al4V alloys which contain aluminum and vanadium responsible for causing serious damage to human tissues, various surface techniques were used on metallic implants [12].

The important methods for surface modification used to coat includ spin coating [13], magnetron sputtering [14], pulse laser deposition [15], laser cladding [16], thermal spraying methods [17], [seeded hydrothermal deposition [18], cold spraying methods [19], laser-engineered net shaping technique [20], laser assisted cold spraying [21]and direct laser melting [16]. These methods depend upon implant type, field of usage and how is the field moving [11].

The processes of several experimental deposition was utilized. One of those that used hydroxyapatite coatings onto Ti6Al4V. Among all surface modification techniques was noted that the sol-gel method enhances the osseointegration and accelerates the healing process [22].

A particular sol spin coating technique used to coat Ti6Al4V surfaces by HAp which was prepared. Coating by spin utilizing to precipitate a regular thin layer on alloy surface used a small amount for coating on the center of surface. Two ways of spinning that utilized either at low-speed—or not. After that along with the high-speed, surface alloy was rotated by centrifugal force of the coating spread on the surface [23].

2. Experimental procedures

This part included, selection of material and preparation samples of Ti6Al4V were processed by using blasting process with particles different dimensions (29 μm, 45 μm, 110 μm, 250 μm) of Al_2O_3 to produced different surface roughness for each sample. After the synthesis of silver doped hydroxyapatite thin films on Ti6Al4V substrate, the samples were characterized by Scanning Electron Microscopy (SEM) and X- Ray Diffraction (XRD). Also, the coatings stability in synthetic body fluid (SBF) and their antimicrobial efficacy were tested.

TEP (triethylphosphite), $P(OCH_2CH3)_3$, ethanol, water deionized, calcium nitrate hydrate($Ca(NO_3)_2 \cdot 4H_2O$), ammonium, and silver nitrate' were used in the preparation of hydroxyapatite with silver, all chemicals where purchased from Sigma-Aldrich Inc., St. Louis, MO.

The solution of silver doped HAp prepared by using of silver nitrate $AgNO_3$ by adding to solution of calcium nitrate $Ca(NO_3)_2 \cdot 4H_2O$ which was hydrolyzed in ethanol CH_3CH_2OH, and stirred for 24 hours then add to mixing of triethylphosphite $P(OCH_2CH_3)_3$ and ethanol deionized H_2O then ammonium NH_4 add as droops to solution until the PH is 9 for solution by using pH paper and allowed to mix for 7 days by magnetic stirrer for stirring the mixing to get the second solution of silver doped HAp.

20 layers of silver doped HAp is deposited onto titanium alloys by spin coater equipment (Laurell model WS-650) at a speed 2000 rpm for 5 seconds. After every deposited layer, the films were dried at 80 °C for 10 min. while, a heat treatment at 500 °C for 30 min was applied and the analysis subsequently is followed as shown in the chart Fig.1 and the code of coated samples obtained with a particles different size of blasting process by using with different sized is shown in table 1.

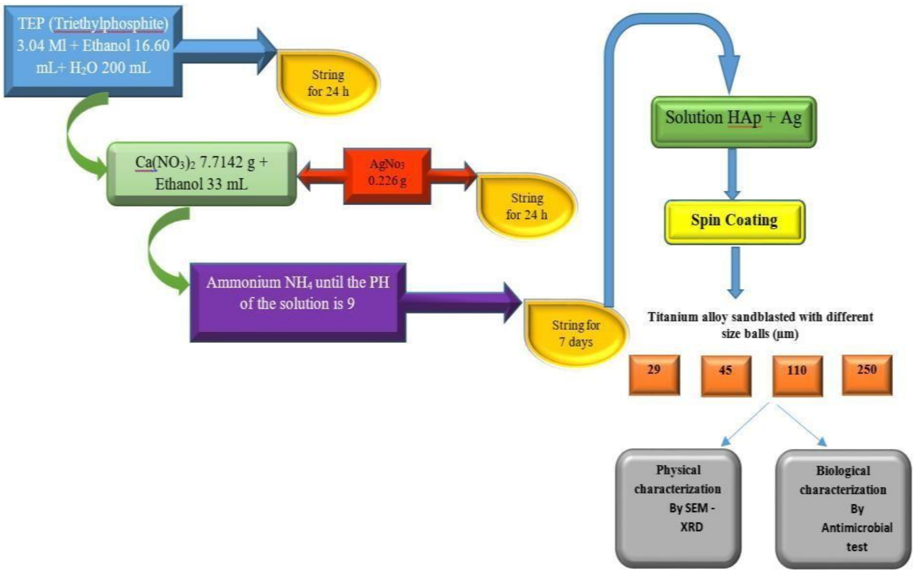


Fig.1. shows the workflow for coating process

Table 1

The code of coated samples

Codification	Alloy	Particle dimension
HAp_Ag_29	Ti6Al4V	29
HAp_Ag _45	Ti6Al4V	45
HApAg _110	Ti6Al4V	110
HAp_Ag_250	Ti6Al4V	250

Films morphology and microstructure were observed with an electronic scanning microscope (SEM) Quanta Inspect F. The images were obtained by recording the resultant secondary electron beam with 30 keV energy. The phase composition was identified using a PANalytical Empyrean model diffractometer equipped with a hybrid monochromator (2xGe 220) on the side and a parallel plate collimator mounted on the PIXcel 3D detector on the diffracted side. Grazing Incidence X-ray Diffraction (GIXRD) measurements were performed at room temperature with an incidence angle of $\omega = 0.5°$ for Bragg angle values of 2θ between 10° and 80°, using Cu Kα radiation with $\lambda = 1.5406$ Å (40 mA and 45 kV).

3. Results and discussions

3.1. X-ray Spectra (XRD)

Necessarily, the surface of roughness for Ti-6Al-4V was created by the processing of abrasive blasting alumina. Roughness substrate boosts the roughness in surface coating. As mentioned by a scholar—significantly, the roughness is considered (on the hydroxyapatite coatings) as a positive impact [24]. The samples of silver doped HAp which are contain HAp_Ag_29 and
HAp_Ag_45 showed in Fig.2. subjected to X-ray spectra analysis.

The result observed a layer of HAp with a rhombohedral structure of calcium phosphate β-$Ca_3(PO_4)_2$ according to ASTM sheets 04-010-0295 for HAp_Ag_45 while tetragonal structure of calcium phosphate, calcium diphosphate $Ca_2(P_2O_7)$ for HAp_Ag_29 according to ASTM sheets 04-009-6231.

The difference in crystallinity which is noted higher for HAp_Ag_29 where the roughness was obtained with 29µm particle than the sample HAp_Ag_45.

While for samples HAp_Ag_110 and HAp_Ag_250 showed in Fig. 1 where the diffraction spectrum shows that the deposition was successful, and in both cases the hydroxyapatite with the hexangular structure of $Ca_5(PO_4)_3(OH)$ according to ASTM sheets' number '00-009-0432'. At the same time, the intensity of the diffraction lines supports the high crystallinity of the samples.

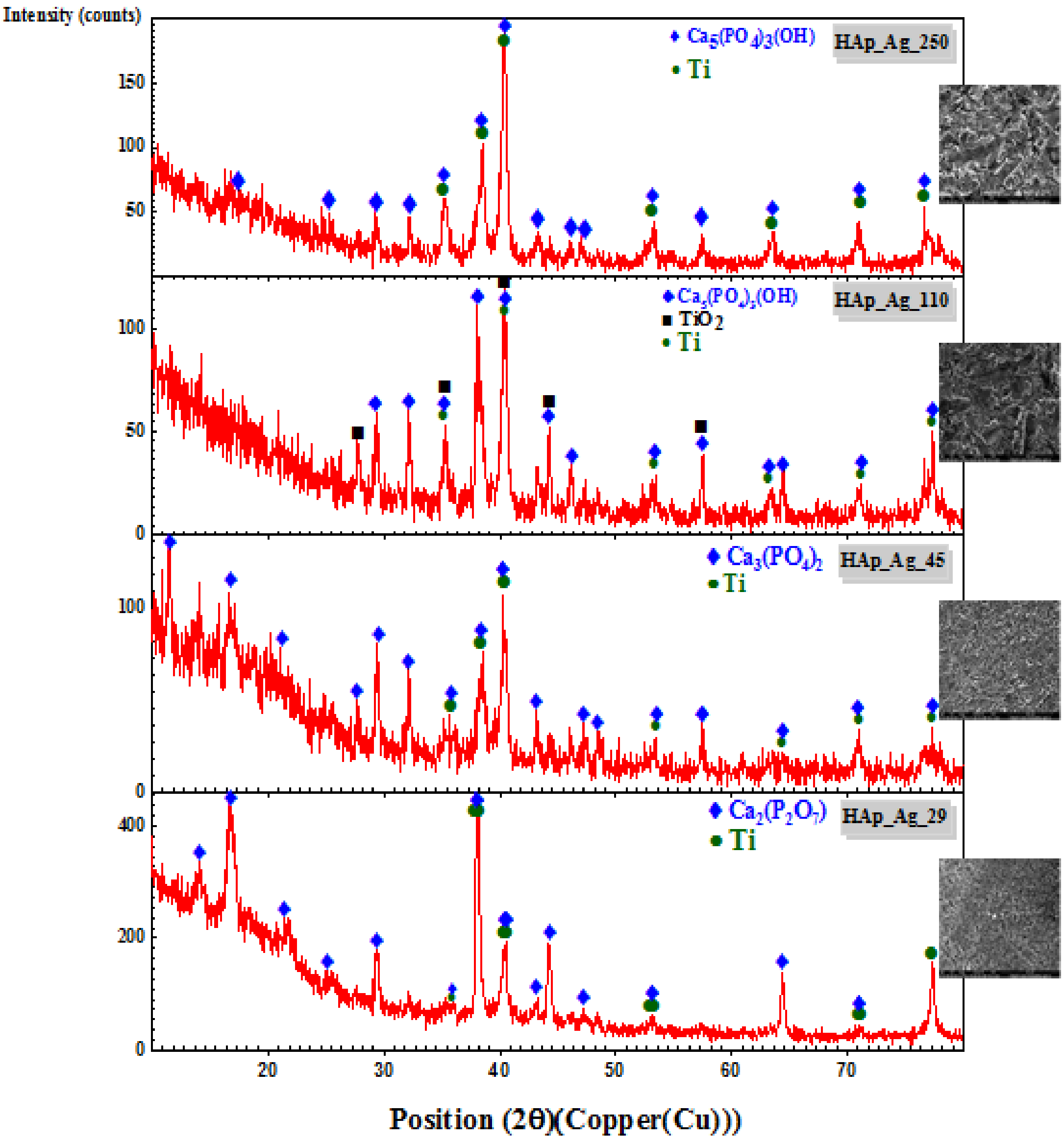


Fig.2 the diffractograms of silver doped hydroxyapatite deposited on Ti6Al4V

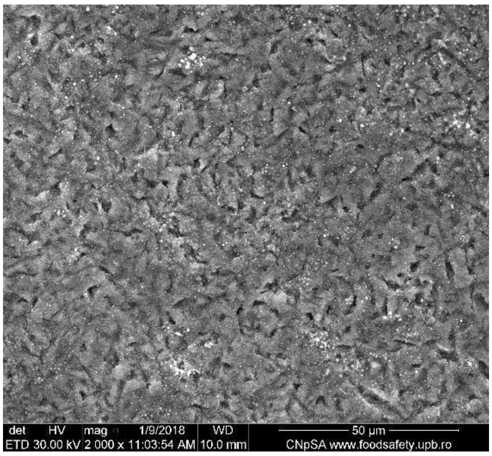
Accordingly, the ASTM sheets' number '00-009-0432' that are built on standardizations, used as substrate for spin-coating deposition. However, it is found out through the analyzed of diffractograms samples, and it has been specified Ag^{+}, it is proved that Ag^{+} entirely substituted by Ca $^{+2}$ in the phosphate ceramics.

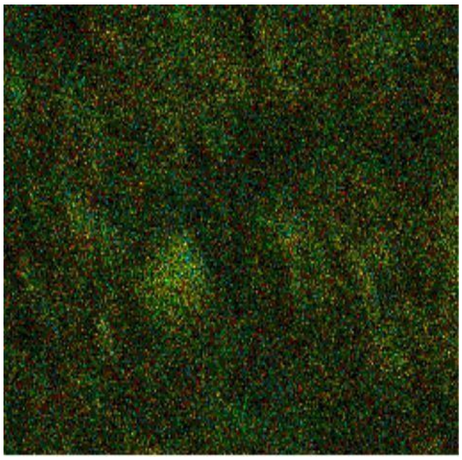
If we make a comparison between a sample of silver doped of HAp with different roughness, It can be noted that calcium diphosphate $Ca_2(P_2O_7)$ structure with 29µm and calcium phosphate β-$Ca_3(PO_4)_2$ with 45µm particle obtained roughness with

tetragonal structure in regard with 29um, and rhombohedral structure in regard with 45um.
However, HAp_Ag_110, and HAp_Ag_250, both cases, the structure is hydroxyapatite with the hexangular structure of $Ca_5(PO_4)_3(OH)$, represented the best status for coating with high crystallinity of both samples.

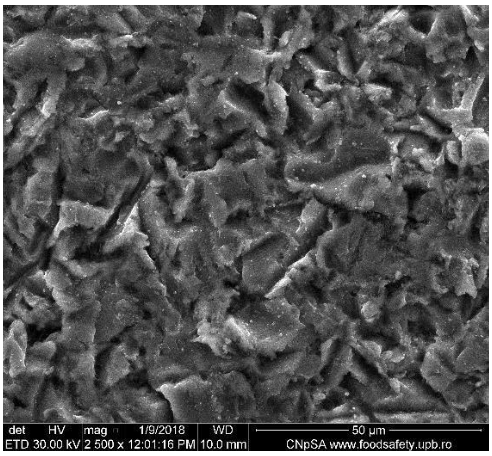
4. Scanning Electron Microscopy (SEM)

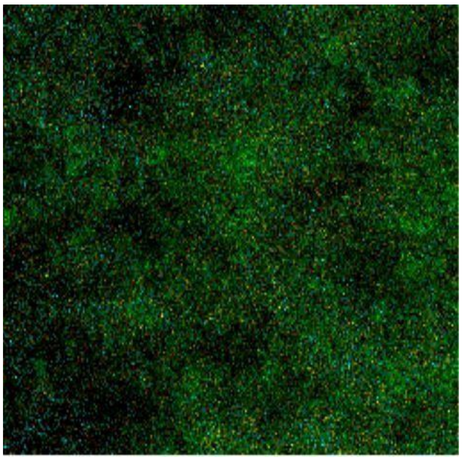
The samples HAp_Ag_29 and HAp_Ag_45 exhibit an ordered abundance of the particulate solution of the deposited layer.
The results of the EDX (Mapping) analysis of each sample confirm that the layer is homogeneously disposed with uniform distribution of specific elements such as calcium, phosphorus, oxygen and silver.
In Fig. 3 are presented the SEM images of the samples containing antibacterial agent (silver) and hydroxyapatite. As it can be seen, the layer consisting in HAp and Ag particles is homogeneously distributed on the Ti surface. The samples HAp_Ag_110 and HAp_Ag_250 have roughly equal proportions of calcium and silver, suggesting that the deposition was carried out
successfully.
Using micrographs, it can conclude that rugosity influences deposition directly. When the roughness is high, the deposition is more successful, and this correlates with the X-ray diffraction analysis. Fig.3 shows SEM of HAp_Ag29 , HAp_Ag_45, HAp_Ag_110 and HAp_Ag_250 .



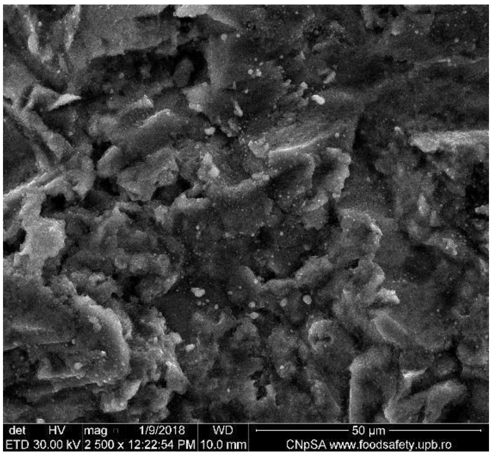


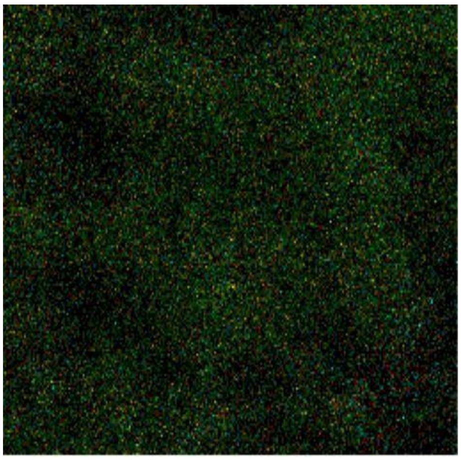
HAp_Ag_29



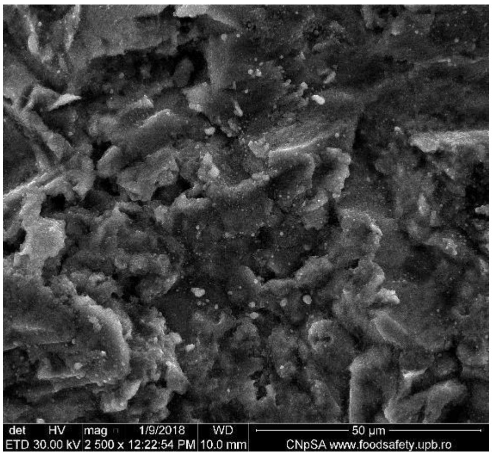


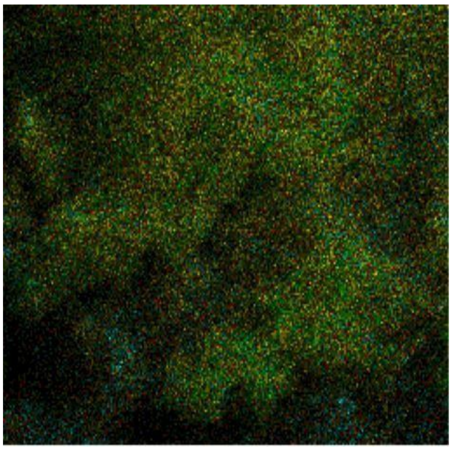
HAp_Ag_45





HAp_Ag_110





HAp_Ag_250

Fig.3 shows SEM images and mapping analysis of the HAp doped Ag coatings

5. Biological characterization

In order to establish the antimicrobial activity of titanium alloys coated with hydroxyapatite and hydroxyapatite with silver, these were tested using the Pseudomonas aeruginosa strain. The numbering of the sample CODs, which shown of the images taken during the biological analysis, is shown Fig. 4.

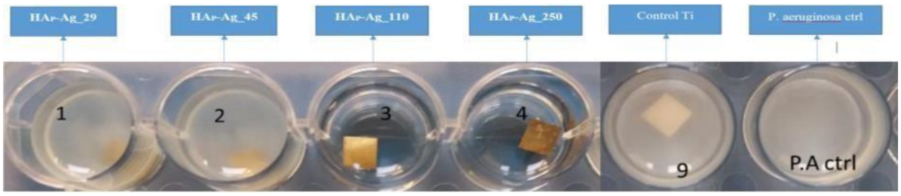


Fig. 4 Microscopic images of the sample CODs

For sample HAp_Ag_ 110 and HAp_Ag_250, no microbial growth was detected visually or spectrophotometrically, which demonstrates the high antimicrobial effect of the obtained materials under Planktonic cultivation- bacterial growth.
After the examination of spectrophotometric, it has been noticed throughout the graph 5, samples 'HAp_Ag_110 and HAp_Ag_250' are the most significant antimicrobial effect on the P. aeruginosa strain. Additionally, the efficacy of the HAp-Ag coatings has been tested and the obtained results are presented in Fig.5.
It was noted that the samples sandblasted with particles of 110 and 250 μm, have positively influenced the antibacterial activity of the proposed coatings with P. aeruginosa strain.
Samples were analyzed to evaluate of biofilm formation, as well as, they were allowed to incubate for 24 hours and subsequently evaluated shows in Fig.6.
It can be seen from the graph that all silver-containing samples showed some inhibitory effect on the formation of monospecific biofilms by P. aeruginosa species, the most significant inhibitory effects of their development being observed in samples HAp_Ag_ 110 and HAp_Ag_250, for the surface of which layers of hydroxyapatite with silver were deposited, finally we observed that all the samples of HAp_Ag have antibacterial properties.
A study showed the usage of different types of bacteria, for the purpose of found out the antibacterial activity for the silver doped hydroxyapatite. Nonetheless, this study appeared that silver existence provides excellent antibacterial property. Conversely, as the increase of silver amount, increase antibacterial [26].

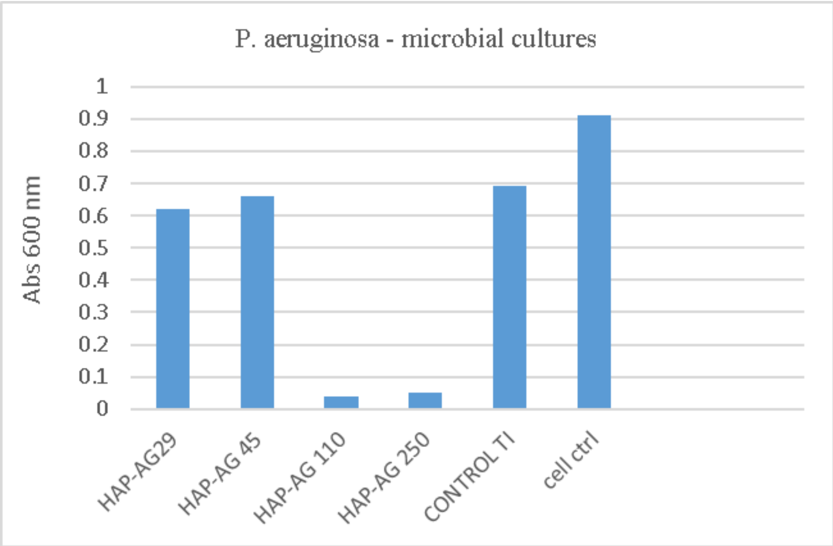


Fig. 5. ABS values at 600 nm suggested the growth of planktonic microorganisms in the presence of Ti6Al4V

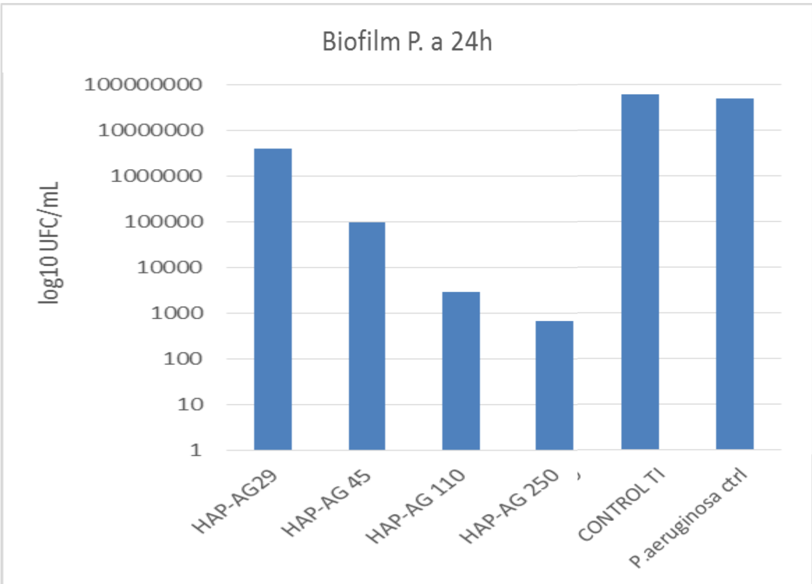


Fig.6. Graphical representation of UFC / mL values representing the development of biofilms on the tested surfaces.

6. Conclusion

The aim was to incorporate Ag into the HAp structure via a natural process that yields bone-like apatite and to obtain a coating with potential antibacterial properties.

The difference in crystallinity which is noted for HAp_Ag_29 where the roughness was obtained with 29µm particleds higher than the sample HAp_Ag_45. Samples containing the antibacterial agent (silver) in combination with hydroxyapatite, demonstrate in the SEM micrographs a layer showing particle
morphologies homogeneously arranged on the surface of the titanium support.

The results of the EDX demonstrated that the samples HAp_Ag110 and HAp_Ag250 have roughly equal proportions of calcium and silver, deposition being successful.

Materials roughness gained by using particles of dimensions 110µm, and 250µm. The affirmative impacts during the antibacterial activity of samples HAp_Ag_110 and HAp_Ag_250 on the P. aeruginosa strain after the spectrophotometric examinations, demonstrated that the samples HAp_Ag_110 and HAp_Ag_250 are the most significant antimicrobial effect on the P. aeruginosa strain, also an inhibitory effect on bacterial action.

The samples HAp_Ag_110 and HAp_Ag_250 is showed that the most significant inhibitory effected about the formation of monospecific biofilms by P. aeruginosa species, on the surface coated with a layer of hydroxyapatite with silver.

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