UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias e Ingenierías

Analysis of a bioactive coating of hydroxyapatite applied by HVOF with an additional layer of chitosan for application in titanium prosthesis.

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Ingeniería Mecánica

Trabajo de integración curricular presentado como requisito
para la obtención del título de
Ingeniero Mecánico

Quito, 20 de diciembre de 2019

UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

COLEGIO DE CIENCIAS E INGENIERÍAS

HOJA DE CALIFICACIÓN
DE TRABAJO DE INTEGRACIÓN CURRICULAR

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Quito, 20 de diciembre de 2019

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RESUMEN

El presente proyecto se basa en el análisis de recubrimientos bioactivos de hidroxiapatita y una capa adicional de quitosano. Estos materiales fueron utilizados son compuestos orgánicos estudiados en el área biomédica. La primera capa de este recubrimiento es la hidroxiapatita, la cual fue colocada sobre la superficie de titanio mediante la técnica de rociado térmico HVOF. Se analizó los parámetros adecuados para el proceso de HVOF teniendo en cuenta una distancia de aplicación de 120mm y la velocidad del alimentador de polvo de 9 rpm, y un total de 30 y 60 pases. Para el recubrimiento de hidroxiapatita se realizó un análisis estadístico de la sección transversal para determinar el espesor obtenido tanto para el recubrimiento de 30como el de 60 pases. Adicionalmente, se implementó una capa de quitosano al recubrimiento la cual se aplicó mediante técnicas de electrodeposición y por simple adsorción. Una vez aplicado el recubrimiento en la superficie de titanio, se analizó y comparó la microestructura de este en diferentes probetas a través del uso de un microscopio de barrido de electrones (SEM). Las probetas con ambas técnicas utilizadas para la adición de quitosano fueron analizadas obteniendo resultados favorables mediante la implementación por electrodeposición aplicando 3V por un tiempo de 20 minutos. Los resultados obtenidos por el análisis de estructura a través del microscopio muestran cambio en la superficie de las probetas lo cual indica que existe adhesión de quitosano a la capa de hidroxiapatita.

Palabras clave: Hidroxiapatita, quitosano, recubrimiento bioactivo, HVOF, prótesis, titanio

ABSTRACT

The present project is based on the analysis of bioactive coatings of hydroxyapatite and an additional layer of chitosan. These materials were used are organic compounds studied in the biomedical area. The first layer of this coating is hydroxyapatite, which was placed on the surface of titanium using the HVOF thermal spray technique. The appropriate parameters for the HVOF process were analyzed considering an application distance of 120mm, the powder feeder speed of 9rpm, and a total of 30 and 60 passes of the HVOF gun. For the hydroxyapatite coating, a statistical analysis of the cross section was performed to determine the thickness obtained for both the 30 and 60-pass coating. Additionally, a chitosan layer was applied to the coating which by two techniques, electrodeposition and simple adsorption. Once the coating on the titanium surface was applied, its microstructure was analyzed and compared in different samples through the use of a scanning electron microscope (SEM). The samples with both techniques used for the addition of chitosan were analyzed obtaining favorable results for the electrodeposition process applying 3V for a time of 20 minutes. The results obtained by the structure analysis through the microscope show change in the surface of the samples which indicates that there is adhesion of chitosan to the hydroxyapatite layer.

Key words: Hydroxyapatite, chitosan, bioactive coating, HVOF, prostheses, titanium

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INTRODUCTION

The need for implants increases with age due to different medical conditions. Studies in this area seek a better biocompatibility and durability of the material implemented in the prosthesis. Parameters such as corrosion resistance and biocompatibility of the material must be analyzed. To improve these characteristics and properties, different coatings or layers are provided to the surface of implants (Bernedo et al., 2019). The composition of the coatings on metal surfaces in prostheses is based on organic materials and its implementation has been studied for several years to improve mechanical and biocompatibility properties, thus improving the quality of life of the patient (Medina-Cruz et al., 2019). Since organic materials are commonly used, the adaptation of the prosthesis to the body is easier, and, the risk of infections due to the prosthesis is reduced. Commonly, titanium is one of the most used materials in prostheses due to its mechanical characteristics similar to properties in human bones such as the elastic modulus (Harun et al., 2018). Also, titanium is used for implant materials due to its corrosion resistance and biocompatibility (Rikhari, Pugal, & Rajendran, 2018). Despite being an extremely important material for prostheses, titanium lacks bone integration properties which is the ability of the material to adapt correctly and completely to the human body (Zuldesmi, Waki, Kuroda, & Okido, 2015). Therefore, to reduce this problem and improve the integration of the prosthesis, it is necessary to analyze bioactive coatings on titanium surfaces with components that allow a better adaptation of the implant to the body.

Hydroxyapatite (HAp), due to its biocompatibility, has been one of the most studied materials in the biomedical area for the last 25 years. The application of this material on coatings for prosthesis surfaces has increased in the last years. An important quality of HAp is that it helps to reduce the risk of local or systemic toxicity thanks to its osteoconductive

properties which promote bone ingrowth (Rincón et al., 2018). In the biomedical area, the osteoconductive properties of coatings are necessary to help to the adaptation of the prosthesis to the body. The importance of HAp is based on the calcium phosphates (CP) that this material contains. These CP ceramics constitute 65-70% of the bone and tooth structure (Kulpetchdara, Limpichaipanit, Rujijanagul, Randorn, & Chokethawai, 2016). For this reason, HAp can be applied in bioactive coatings to improve its properties. An important aspect to consider is the calcium-phosphate (Ca/P) ratio since it influences the integration and stability of the compound in the human body (Kulpetchdara et al., 2016). Studies show that it is necessary a Ca/P ratio greater than 1.0 to have a good stability of the compound; these researcher determine that the Ca/P ratio of HAp is 1.67 which makes this material viable for biomedical applications (Kulpetchdara et al., 2016). Despite the importance of this material, the implementation of pure HA in coatings is not feasible since a greater biological activity is required. This biological activity, as mentioned before, allows a better adaptation of the patient to the prosthesis. For this reason, compositions of organic materials with hydroxyapatite or complementary coatings placed on the HAp layer are studied to improve biological and mechanical properties (Su, Li, Zhang, Wang, & Zhang, 2018). As mentioned previously, hydroxyapatite is a material used for bioactive coatings on metal prostheses, which include hip titanium implants. In the last 25 years HAp has been studied since it has a great integration in the body that promotes the formation of a layer in the surface of the prosthesis with properties and composition similar to those of the bone structure. Thanks to this layer, the patient can have a better and faster adaptation to the prosthesis (Rincón et al., 2018) and also, the risk of infections is reduced. For the obtainment of this compound, the synthesis and characterization of hydroxyapatite has been studied for several years. Last researches have focused on low-cost sintering of HAp through

natural sources, using bovine or fish bones that are commonly discarded materials. The use of this organic waste also represents a great economic and environmental advantage (Rincón et al., 2018). The process applied or used has an impact on the quality and crystallographic structure (Ferro & Guedes, 2019) of the HAp powder obtained. Commonly, mechano-chemical methods are used for the synthesis of hydroxyapatite powder, optimizing crystallographic properties and, in this way, improving biological performance on the coating applied to the implant surface (Ferro & Guedes, 2019).

On the other hand, chitosan is a natural compound abundant in nature. This cationic polysaccharide is studied in different fields such as tissue engineering, bioactive coatings (Avcu et al., 2019), among other applications. This material is generally obtained from shrimp shell which makes it an attractive compound since the obtaining cost is low (Avcu et al., 2019). Chitosan is a polymeric compound that is studied in the medical area due to its osteoconductive properties, biodegradability, biocompatibility, bio-functionality, among other characteristics (Bravo-anaya et al., 2019). Generally, this compound is added to coatings on titanium surfaces by electrophoretic methods (EPD) which consist on a solvent with dispersed particles surrounded by an electric field between two electrodes (Avcu et al., 2019). EPD allows the formation of a uniform layer of chitosan on the surface, thus, mechanical properties, adhesion , micro porosity, among others, are improved, in this way, it is possible to prevent crack formation (Karimi, Kharaziha, & Raeissi, 2019) on the coating. In addition, it is possible to add more composites to the coating in order to improve the mechanical and biological properties. For the moment, studies have been carried out with HAp and chitosan compositions for coatings in the implementation on titanium surfaces by electrodeposition (Redepenning, Venkataraman, Chen, & Stafford, 2003) due to the versatility of the method. This method

allows a better distribution of the composites among the coating layer on the surface and the cost involved is accessible. The current applied during electrodeposition method also allows to obtain different layer thickness depending on the materials worked.

Electrodeposition is a method widely used and studied since the 90s (Redepenning et al., 2003) due to its versatility and ease of implementation for coatings on metal surfaces. This method is based on a solution, or electrolyte, with dispersed material particles, which, by the application of an electric field, form a thin layer on the metal material that is being coated (Wang et al., 2013). The advantages granted by this method are, as mentioned before, ease of implementation, reduced procedure time and the ease of working at room temperature. For these reasons, electrodeposition is a technique widely used in areas such as biomedical and biotechnology (Wang et al., 2013). Therefore, in this research, electrodeposition is used for the addition of a chitosan layer to the HAp coating on the titanium surface. During the electrodeposition process, the particle size is important in order to have a better bonding to the surface of the coating. As shown in Figure 1, the particles dispersed in the electrolyte occupy the empty spaces of the surface, creating a thin layer. For this process, is important to determine the current that must be applied based on the materials which are being worked (Redepenning et al., 2003), in this way, the layer formed will be homogenous and coating properties will improve.

The methods for the addition of coatings to the titanium surface can be carried out differently, for instance, the application of hydroxyapatite in titanium implants can be made by thermal spray (TS) methods, which allow a better fixation of the coating to the surface, thus extending the useful life of the prosthesis and guaranteeing the life quality of the patient (De Vizcaya-Ruiz et al., 2018). Surface finish and textures of the implant directly affects bond

strength coatings, having a direct influence on adhesion, crack formation and delamination of the coating (Levingstone, 2008), for instance, TS methods are analyzed for the application of these coatings. One of the major disadvantages with thermal spraying methods is the work temperature since at high temperatures, formation of undesired secondary phases in the HAp coating can be generated (De Vizcaya-Ruiz et al., 2018). To avoid these inconveniences, the High Velocity Oxy-Fuel (HVOF) thermal spraying technique can be used. This technique consists in the deposition of HAp particles at high speed on a surface by the combustion of a flame with a temperature lower than 3200˚C (Henao et al., 2018). An important parameter to be considered in HVOF process is the oxygen-fuel relation since it affects to the behavior of the hydroxyapatite particles and its capability of melting and distribution among the surface (Kulpetchdara et al., 2016). Another parameter to consider in the application of HAp by HVOF method is the particle size since it affects directly to the adhesion and distribution of the layer among the surface, increasing the probability of crack formation or delamination (De Vizcaya-Ruiz et al., 2018).

At the moment, there are studies for the implementation of coatings of HAp and chitosan, however, the techniques that have been used for their application are different from the ones proposed. Previous investigations have been based on the application of nanocomposite coatings of hydroxyapatite and chitosan using electrophoretic techniques which consist on the application of a magnetic field (Pang & Zhitomirsky, 2007). Thanks to the study of these coatings it has been possible to determine the improvement in the corrosion resistance of the implant as well as the biocompatibility of the coating. The analysis of the proposed coating is important since its materials can be obtained by low cost techniques. Additionally, the processes used for the addition of the coating are optimal to preserve its mechanical

characteristics and thus ensure adhesion to the prosthesis and adaptation to the body. Also, the increase of bioactivity in the coating is important to the adaptation of the prosthesis. For instance, the aim of this research is to combine the materials such as chitosan and HAp to provide better properties to the coating, guaranteeing the patient's life.

METHODS

This section discusses the methods as well as the materials used at each stage of the project. It begins with the explanation of the preparation of the titanium samples prior to the application of the HAp coating layer. Then, the parameters followed for the High Velocity Oxy-Fuel (HVOF) process in the implementation of the first coating layer are verified. In addition, the methodology followed for the analysis of this coating is shown prior to the addition of a chitosan layer. Finally, the methodology for increasing the chitosan layer to the coating and its proper analysis is described.

Sample preparation

The analysis of the coating of this Project is made on a titanium surface for which, a ½ in diameter titanium bar is required. This bar is cut with a precision saw (BUEHLER IsoMet 1000 Precision Saw), each test piece with a length of 8 mm so that they can be placed in the sample holder designed for the HVOF process. Additionally, to increase and improve the adhesion of the coating to the base material, it is necessary to increase the surface roughness. For this reason, a sandblasting process is carried out with aluminum oxide powder, Al_2O_3, which allows to increase the surface roughness of the titanium and improve the process. The parameters for this process are summarized in Table 1. The selection of the aluminum oxide for the sandblasting was based on previous investigations which have determined the optimal material to be used.

Table 1 *Parameters for the sandblasting process*

Application distance	100 mm
Time	10 s
Compressor pressure	105 MPa
Sandblasting material	Aluminum oxide, Al_2O_3

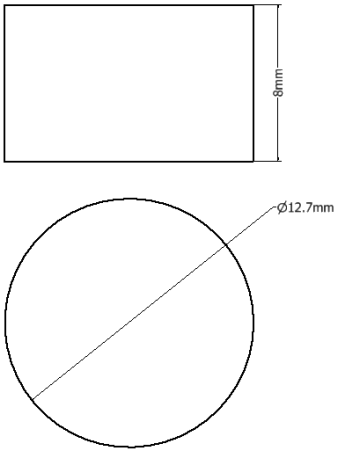

Figure 1 *Titanium sample dimensions*

Additionally, the roughness of the sandblasted surface of the samples is measured with a roughness meter to verify that the process has been applied correctly. The approximate value of the desired surface roughness, based on previous research is in the range of 3 to 5μm and this should be verified with this measurement. It should be considered that sandblasting is an abrasive process that can leave impurities on the surface of the titanium which affect the coating, so it is necessary to go through an ultrasonic cleaning process using a BUEHLER UltraMet 2002 Sonic Cleaner machine. In this machine, the samples were immersed in alcohol and cleaned for 15 minutes. After this cleaning process, the specimens were dried and placed in the holder to allow the addition of HAp through HVOF.

Figure 2 *Sample holder for the HVOF process*

High Velocity Oxy-Fuel process

The High Velocity Oxy-Fuel, HVOF, is a thermal spray process that is based on the release of particles from a gun at high speed, these particles collide with the substrate to be deposited. The temperature of the flame with which these particles are deposited causing them to merge so that they are malleable and their adhesion to the substrate improves. The process is performed with two gases for the flame, the first is oxygen and the other gas used for this investigation is propane. The combination of both gases helps to form the flame of the HVOF gun. In this investigation, hydroxyapatite powder particles were used to deposit on the titanium substrate and thus generate a coating of this material on the specimens for their respective analysis. In this research, the gun used for the HVOF was a DiamondJet 2600 which has a water-cooled front that allows the particles to leave with a much higher speed. Depending on the quality of the coating wanted, different fuel gases are used in the process, in the case of this investigation fuel gas is propane. As previously mentioned, this gun is connected to a manual device with which the HVOF procedure is performed for the addition of HAp on the titanium surface. Additionally, it must be checked that the pressure of both oxygen and propane tank is not less than 3bar to ensure the fluidity of the powder during the process.

Hydroxyapatite layer addition

As described before, the implementation of the coatings can be done by different methods, including thermal spraying for better adherence to the metal surface. For the application of HAp by HVOF, it is necessary to consider parameters such as application distance and preheating temperature, these parameters are described in Table 1. It should be noted that, for a greater fluidity of the HAp powder in the feeder, it is necessary to sift it up to 10 um, this ensures that no large agglomerates pass through the machine and to improve the flow of powder to the gun. Prior to this process, it is important to keep the powder at a temperature of approximately 150 ^{0}C to reduce its humidity and increase its fluidity. Leftover lumps from the sifting process are separated for further treatment. The HVOF process was made with a Diamond Jet 2600 gun, since this process is performed manually, without the help of a robotic arm, the velocity should be as constant as possible to ensure that the coating is uniform throughout the entire titanium surface.

Table 2 *Parameters for the HVOF process*

Preheat powder temperature	150 ^{0}C
Preheat feeder temperature	100 ^{0}C
Preheat sample temperature	50 ^{0}C
Application distance	200 mm
Feeder velocity	9 rpm

The HVOF process is applied to a total of 14 samples. A total of 7 specimens are coated with 30 passes of the gun, while a total of 60 passes are applied to the remaining 7 samples. In both cases the same parameters previously described are maintained.

Figure 3 *HAp addition by thermal spray HVOF process*

HAp coating thickness analysis

It is important to analyze the cross section of the samples to determine the average thickness of the hydroxyapatite layer obtained with both 30 and 60 passes of the HVOF gun. Some images are found in the SEM and are used to obtain data for a statistical analysis of the coating thickness along the samples. The ImageJ software is used to find the thickness data, the steps for setting this software are presented in the Annexes section. With the length data in μm found with ImageJ, the values are imported into MiniTab to perform the statistical analysis of the coating thickness.

Chitosan solution preparation

With the HAp layer on the titanium surface, it is possible to add a chitosan layer to improve the bio-functionality of the coating. The first method used is electrodeposition in which the electrolyte contains dispersed chitosan particles. The chitosan solution is prepared in an iron (MTOPS MS300HS) that keeps the temperature constant throughout the process. In a beaker 0.5 g of chitosan and a 0.25% solution of acetic acid, CH_3COOH, dissolved in distilled water are added. The solution is left on the plate for 30 minutes, it is important the addition of

a stirrer to ensure homogeneous mixing of the components, as shown in figures. The parameters and solutions used in the preparation of the chitosan solution are shown in Table 3.

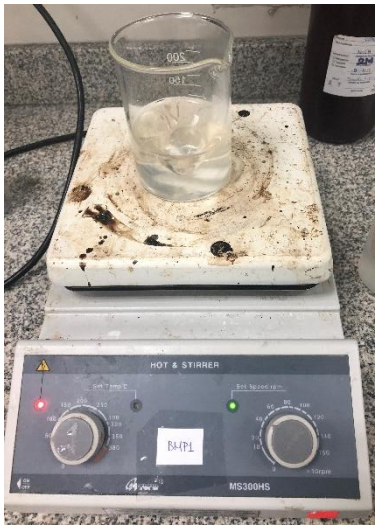

Figure 4 *0.5% Chitosan solution preparation*

Table 3 *Parameters used for the 0.5% Chitosan solution*

Heater temperature	150 ^{0}C
Chitosan	0.5 gr
0.25% Acetic Acid (CH_3COOH) Solution	2.5mL CH_3COOH in 100mL of solution

**Note: These parameters are used for 100 mL of 0.5% chitosan solution*

The 0.5% chitosan solution prepared must be cooled to measure the pH, this value has to be close to or equal to 5.5, so it is necessary to titrate the solution until it reaches the required pH. Sodium hydroxide, NaOH, 2M is used for this process. This solution is added to the chitosan gradually with a micro-pipette, measuring the pH after each addition of NaOH, until it reaches the value of 5.5. Once the solution is titrated, it must be filtered to remove agglomerated gels which are generated in the process and that affect the correct electrodeposition of chitosan. A vacuum pump is used in the filtrate of the solution and it is

important to emphasize that approximately half of the prepared solution is lost in this process. After the filtration, the chitosan solution can be stored or used for the electrodeposition.

Chitosan layer addition by electrodeposition

For the electrodeposition, the titanium sample with the HAp layer is immersed in 50 mL of 0.5% chitosan solution, and a platinum electrode is used, as shown in Figure 5, while a constant current and voltage are applied for 20 minutes. The parameters for the electrodeposition are described in Table 4.

Table 4 *Parameters for the Chitosan electrodeposition*

Voltage	3V
Current	0.02 A
Time	20 min
Electrolyte	0.5% Chitosan solution
Electrode	Platinum

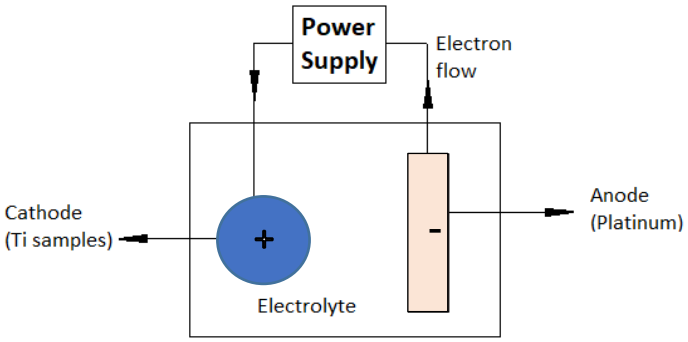

Figure 5 *Basic diagram for chitosan electrodeposition*

After electrodeposition, the samples must be stored and labeled correctly to undergo a lyophilized process prior to the analysis of the coating properties

Chitosan layer addition by simple adsorption

The chitosan particles form an additional thin layer to be analyzed. To determine the proper procedure for the addition of the chitosan layer, certain samples are prepared by adding this layer by simple adsorption. This technique is based on adding 30 μL of 0.5% chitosan solution on the surface of the HAp coating and leaving the sample for 20 minutes in a vacuum chamber (BUEHLER CastN' Vac, Castable Vacuum System) at a pressure of -0.5 bar, so that it adheres correctly to the previous coating layer. After this process, the samples are correctly stored and labeled for the lyophilized, previous to the SEM analysis. The thickness and efficiency of the simple adsorption process can be compared with the electrodeposition made in different samples and therefore, determine the preliminary results in the process for the addition of the chitosan layer.

Sample analysis

In the addition of the HAp layer by HVOF, samples with a medium and a thick coating compared on the influence on the chitosan layer. The analysis is performed for HAp-coated samples, and also for the HAp-Chitosan coating system with electrodeposition and simple adsorption. The metallographic analysis of the samples require each one of them to be prepared correctly for the analysis in SEM (JEOL JSM-IT300LA). For the cross section analysis of the coating, each specimen is mounted in epoxy resin and placed in the vacuum chamber (BUEHLER CastN' Vac, Castable Vacuum System) for 20 min in order to reach better results. Each sample is rough and polished enough to analyze the coating. The data obtained for the thickness and distribution of the coating with the two techniques used for the addition of chitosan can be compared to determine the appropriate procedure.

RESULT ANALYSIS

This section reports the results obtained from the partial and total coating analysis as well as the characterization of the HAp powder. For the coating, the thickness of the hydroxyapatite layer was analyzed for both 30 and 60 passes. The coating is then studied with the addition of chitosan, the results are presented for both electrodeposition and simple adsorption. Additionally, the statistical analysis of thickness on each analyzed coating is provided to determine the most viable procedure for its application.

Particle size

This analysis begins by verifying the size of the HAp particle. The powder is analyzed by SEM, and as it is seen in Figure 6, these particles are quite amorphous. The morphology of the particles is important since by making them spheres, processes such as HVOF can be significantly improved. Despite the morphology of the HAp particle, several samples of the powder were analyzed and images were obtained to perform the statistical analysis with the ImageJ and Minitab software, giving the following results.

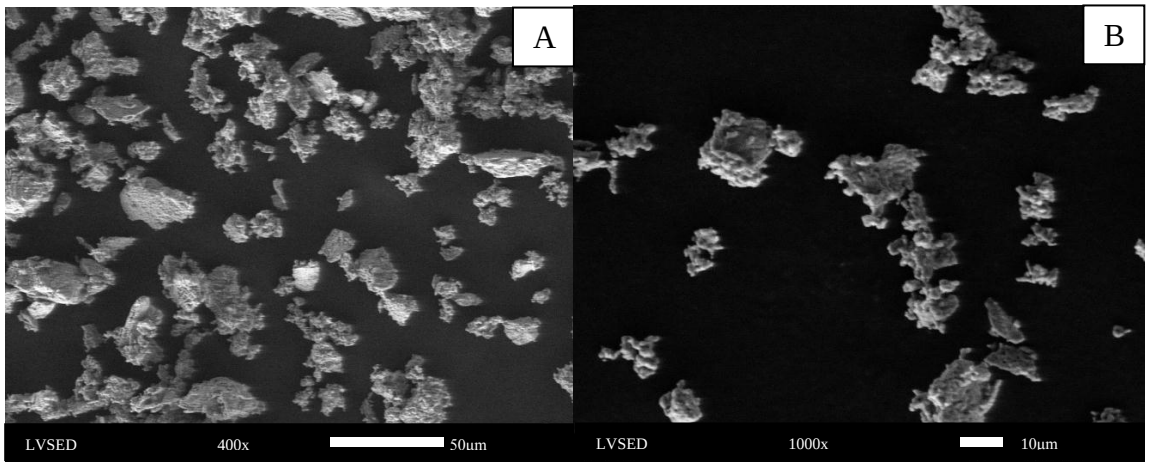

Figure 6 *SEM photos of the HAp powder with a magnification of A) 400x, LVSED Calibration bar: 500µm, B)1000x, LVSED, Calibration bar: 10µm*

Table 5 *Statistical analysis for the HAp powder particle size*

Mean	28.308 μm
Min	12.044 μm
Max	48.694 μm
StDev	8.196

Hydroxyapatite coating analysis

The SEM analysis began with the visualization of the coating morphology to verify the existence of a HAp layer on the previously prepared titanium surface. This can be compared with the sandblasted surface at the same magnification. The results of this analysis are found in Figure 7. With this visualization it can be verified that the surface of the material was completely covered, however it was necessary to analyze the thickness of the coating obtained for both 30 and 60 passes of HAp powder. Additionally, in the cross-sectional analysis of the coating was important to verify adhesion to the substrate and also between HAp powder layers.

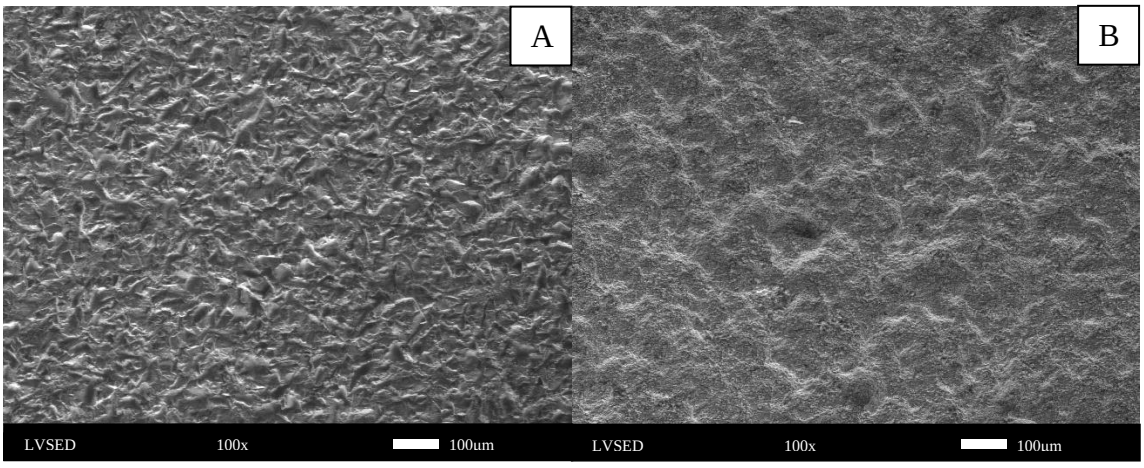

Figure 7 *SEM photos of the simple Surface with a magnification of 100x LVSED A) sandblasted surface, B) HAp coating. Calibration bar: 100μm*

30-passes HAp layer thickness analysis

To analyze the final thickness of the coating for the 30-pass process of the HVOF gun, the sample was prepared and mirror-polished. This mirror-polish was required for a simpler and more precise analysis. The samples used are shown in Figure 8 and the coating analyzed is found on top of the sample as shown. The coating of all samples were seen in the scanning electron microscope, SEM, to make a later comparison with different thickness of the HAp layer.

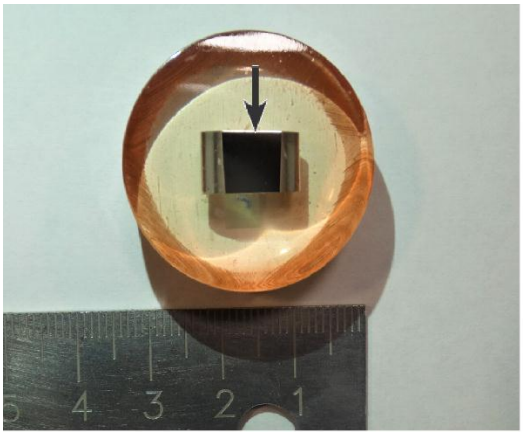

Figure 8 *HAp layer cross section*

In the cross section analysis, as shown in Figure 9 it is noticed some cracks in the interface of the HAp coating with the titanium surface, so that the adhesion of the coating may not be optimal. These cracks should be avoided to prevent the delamination of the coating. Also, it is important to study the parameters for the preheating temperature on the substrate in order to avoid separation between the titanium and the hydroxyapatite. Along the surface, intermediate cracks appear in the coating, these must be considered to benefit the bonding with the next layer of chitosan. On the other hand, HAp powder spheres can be noticed throughout the coating, so the particles did not melt completely, creating only layers on top of each other not having a proper adhesion.

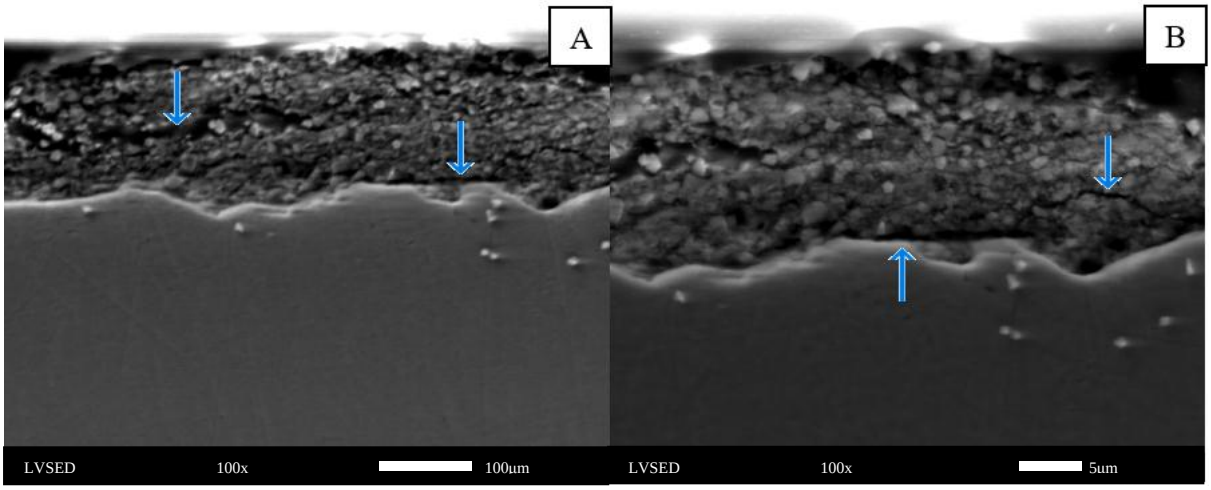

Figure 9 *Cross section HAp coating with 30 passes with magnification of A) 2000x, LVSED, Calibration bar: 10µm, B) 2700x, LVSED, Calibration bar: 5µm*

The results for the statistical analysis for the thin HAp coating are shown in Table 6. Also, the data distribution is plotted in a histogram to analyze the coating thickness uniformity along the sample surface.

Table 6 *Statistical analysis for the HAp layer of 30 passes*

Mean	14.087 µm
Min	5.876 µm
Max	23.502 µm
StDev	3.705

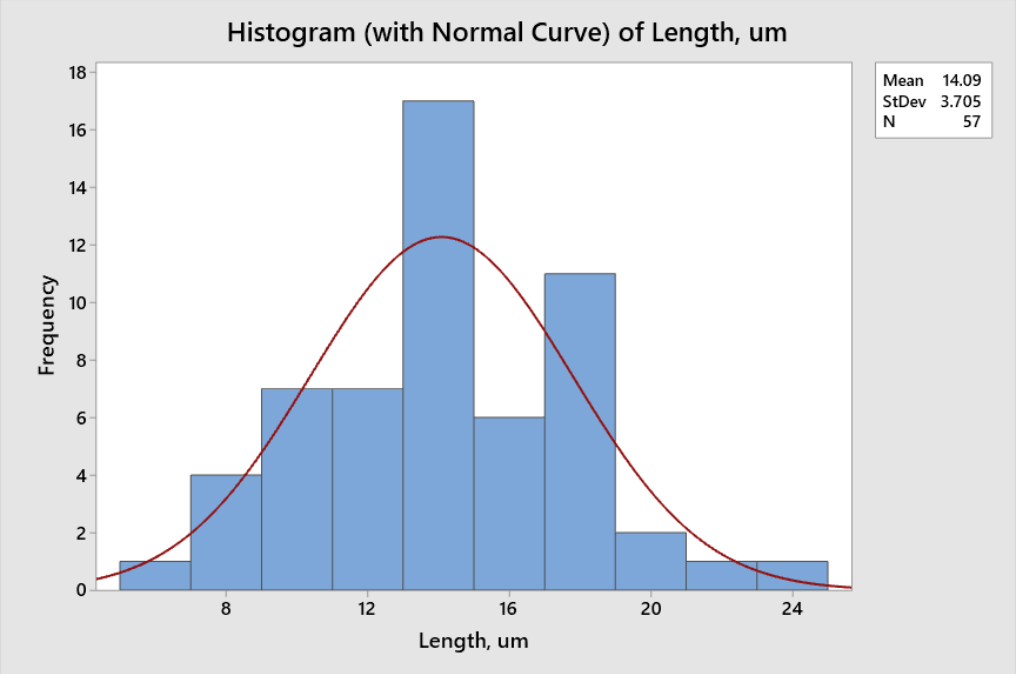

Figure 10 *Histogram of data analyzed for the HAp layer of 30 passes*

With the statistical analysis it was determined that the coating thickness for 30 passes is approximately 14 μm. In the histogram of Figure 10 it can be seen that the greatest distribution of thickness data is found in a range of 13 μm to 15 μm, however, it can be noted that the distribution of the coating is not completely uniform throughout the surface.

60-passes HAp layer thickness analysis

In the coating analysis for the hydroxyapatite layer with 60 passes, the same procedure previously described was performed. The titanium samples were mounted in epoxy resin and mirror-polished. The photos obtained in SEM are shown below.

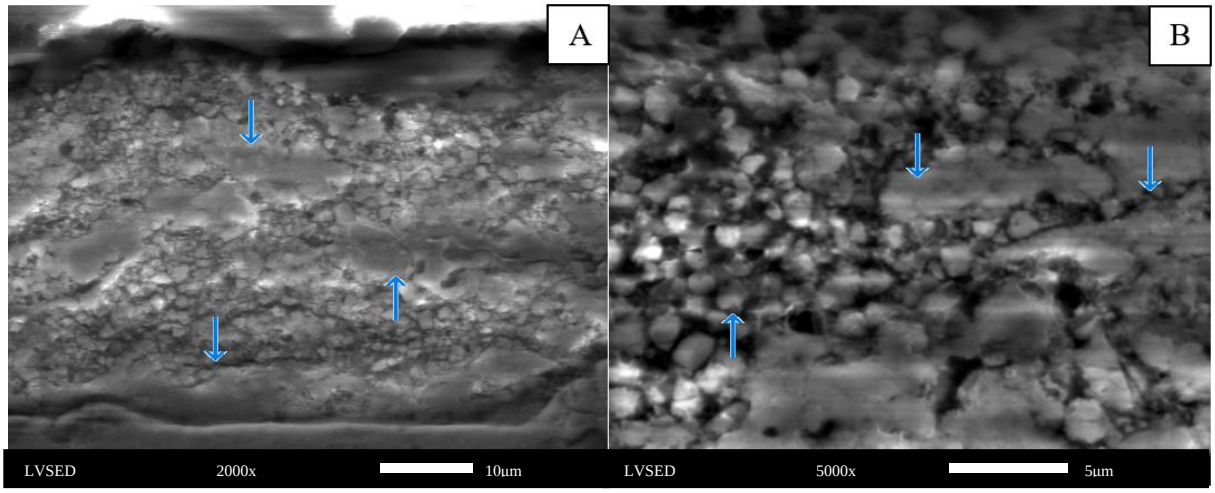

Figure 11 *Cross section HAp coating with 60 passes with magnification of A) 2000x, LVSED, Calibration bar: 10µm, B) 5000x, LVSED, Calibration bar: 5µm*

For the statistical analysis the methodology described previously was followed, and the results obtained are presented in Table 7. On the other hand, it can be seen in the histogram of Figure 12, the data for the coating thickness for the thick HAp layer is better distributed compared to the data for the thickness of the thin coat of HAp. This could also be noted in the images obtained in SEM for the cross section of the coating. Despite this, it is noticed areas with the thinner coating along the surface but less frequently.

Table 7 *Statistical analysis for the HAp layer of 60 passes*

Mean	35.569 µm
Min	23.232 µm
Max	41.798 µm
StDev	3.479

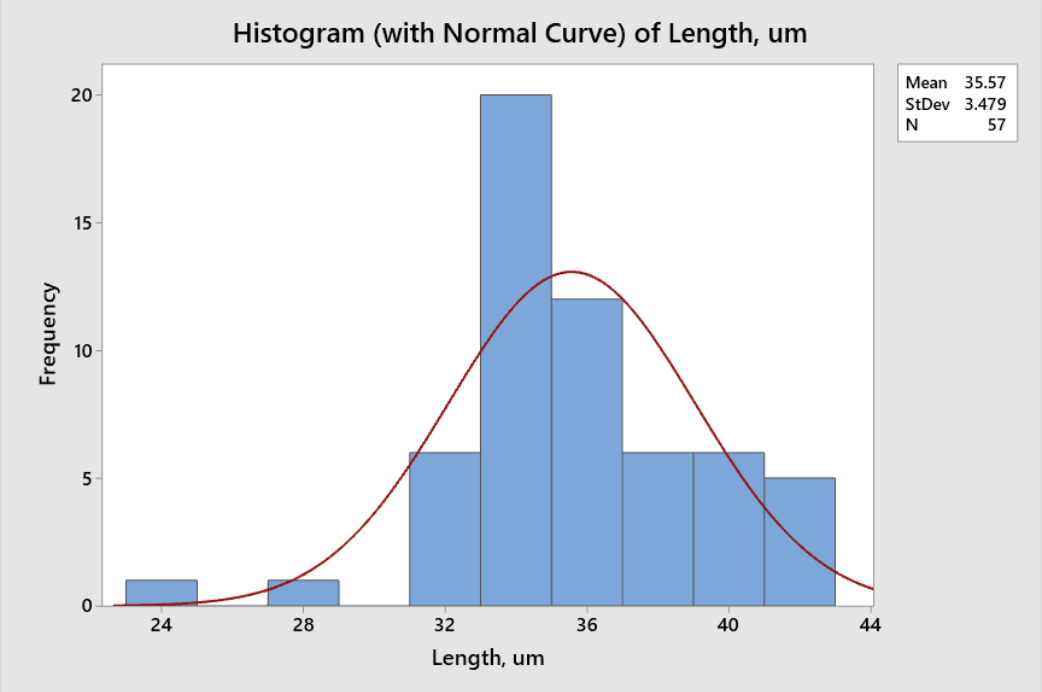

Figure 12 *Histogram of data analyzed for the HAp layer of 60 passes*

In Figure 11 which shows the cross section of this coating it can be seen areas in which there is a better fusion of HAp particles, however, there are cracks along the coating that, as previously mentioned, can benefit the addition of the chitosan layer. Based on previous investigations, cracks and pores are favorable for the multi-layer-coating bonding. Additionally, it is known that the roughness and porosity of the coatings helps the bone integration process of the prosthesis to the patient. As can be seen in the images found of the coating, several porosities and cracks are found along the surface and cross section. These imperfections favor the adhesion of the second coating layer which makes it have a better stability and runs a lower risk of delamination inside the body (De Vizcaya-Ruiz et al., 2018).

On the other hand, there is also a better adhesion to the surface of the titanium although the same preheating and sample preparation parameters were used. With the statistical analysis of the thickness of the coating it can be concluded that it has a greater uniformity along the sample, however there are areas in which the coating is thin.

Comparison between thin and thick HAp layer

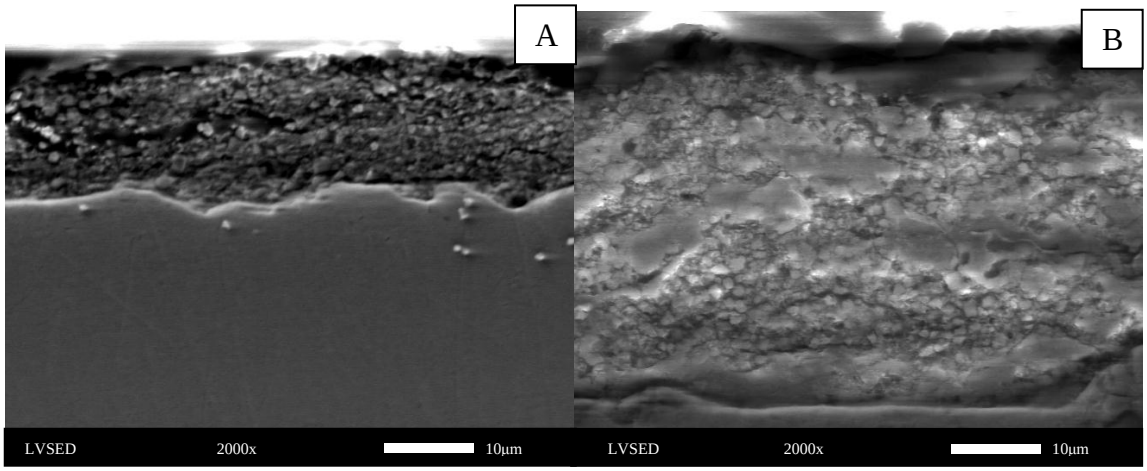

Figure 13 *Cross section HAp coating with magnification of 2000x for A) 30 passes B) 60 passes. LVSED, Calibration bar: 10µm*

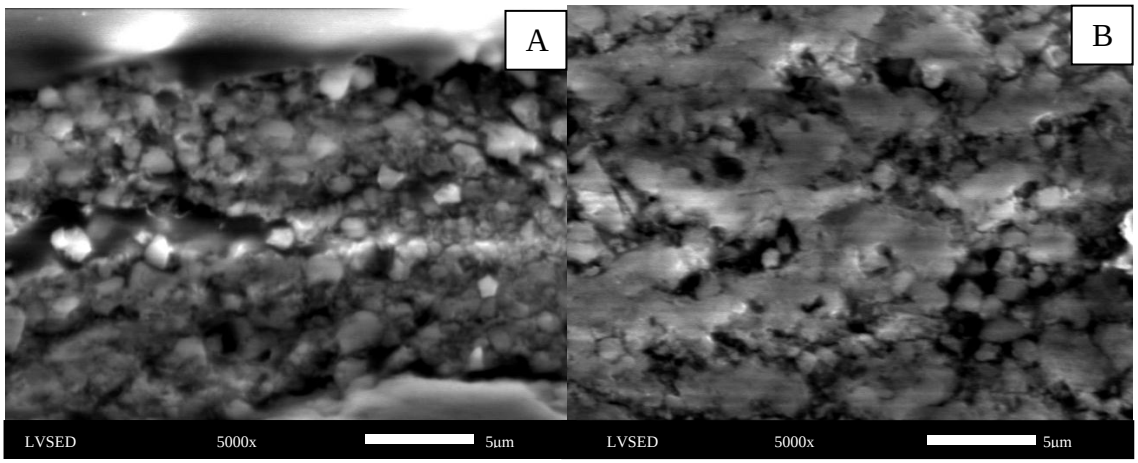

Figure 14 *Cross section HAp coating with magnification of 5000x for A) 30 passes B) 60 passes. LVSED, Calibration bar: 5µm*

In the figures presented, the differences previously described for the two coatings can be compared. The difference in the thickness of the HAp coating and the areas with the highest melting of particles for the thick coating can be clearly seen. Additionally, X-Ray Diffraction (XRD) tests are required to verify that these fusion of HAp particles do not cause fragility of the coating since it can easily be delaminated.

Chitosan coating analysis

For the chitosan coating analysis it was necessary to perform a surface inspection to verify the chitosan adherence to the HAp coating. The results for both, electrodeposition and simple adsorption are presented in this section. Additionally, the results for each method are compared.

Electrodeposition.

The procedure started by analyzing the surface of each sample to verify the addition of chitosan. In figures above the surface at different magnifications can be compared for both, thin and thick HAp coating layers with the addition of chitosan by electrodeposition.

Thick HAp coating layer.

The figures above show the chitosan deposition on the thick HAp coating layer. In Figure 15 the surface area of HAp is compared with the surface of the chitosan added by electrodeposition, however an appreciable difference cannot be noticed at a low magnification. For this reason, a higher magnification analysis was performed to verify surface changes.

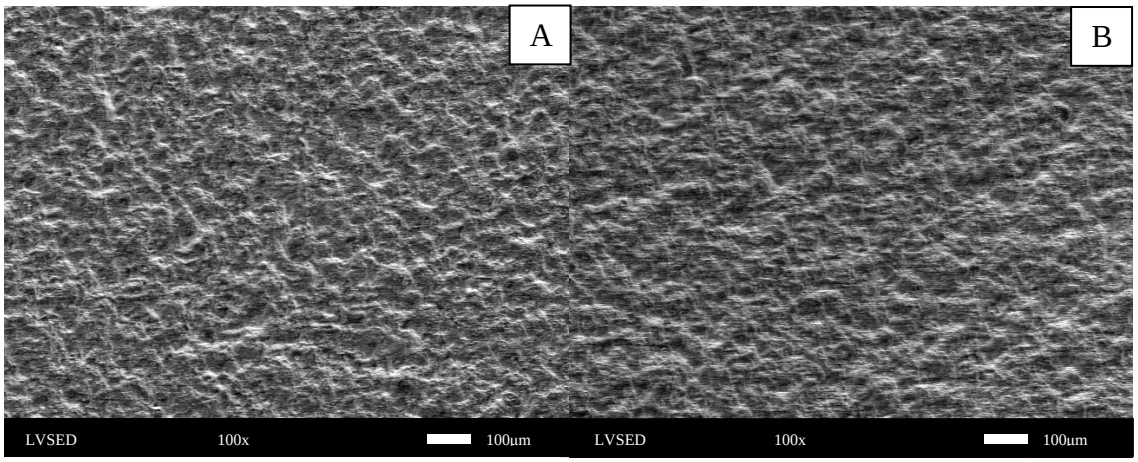

Figure 15 *Surface analysis with a magnification of 100x for A) thick HAp coating B) electrodeposited chitosan. LVSED, Calibration bar: 100µm*

With a magnification of 500x it is possible to appreciate certain changes in the analyzed surface. In Figure 17, it can be compared that on the surface of hydroxyapatite there are certain areas with greater agglomeration of particles, comparing this surface with that of electrodeposited chitosan the presence of a thin layer of this compound was verified. In Figures 17 and 18 it can be seen that in the chitosan layer there are areas with holes, this could have been due to the presence of bubbles by the same electrodeposition process which leave these holes on the surface of the coating. Also, in these figures it can be noticed that the chitosan coating is also irregular throughout the surface. There are certain areas more elevated than others, this effect could be produced also by the irregularity of the HAp coating layer on the titanium surface.

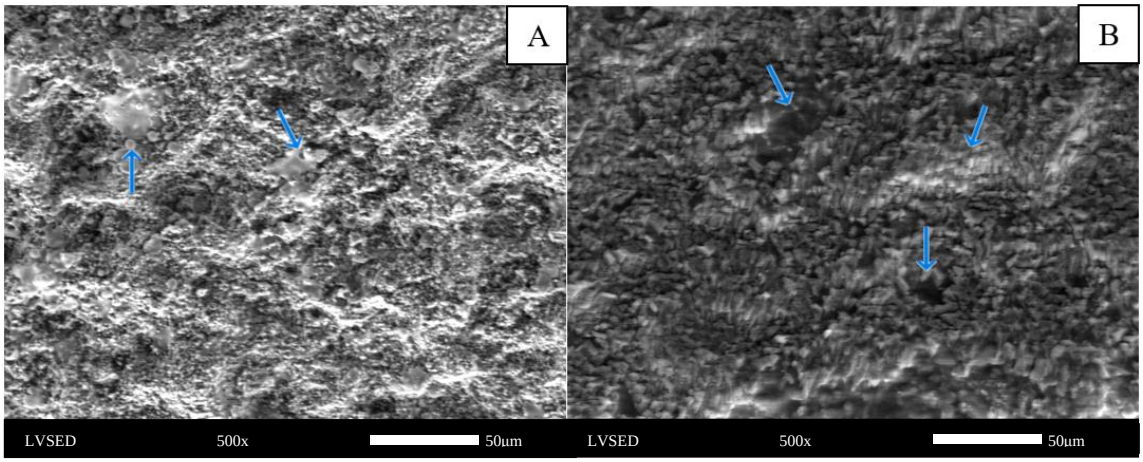

Figure 16 *Surface analysis with a magnification of 500x for A) thick HAp coating B) electrodeposited chitosan. LVSED, Calibration bar: 50µm*

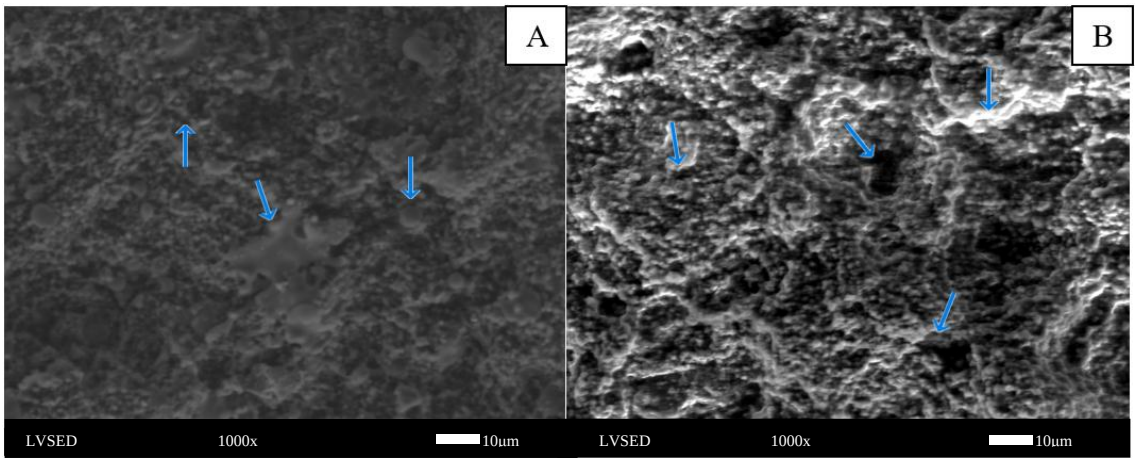

Figure 17 *Surface analysis with a magnification of 1000x for A) thick HAp coating, B) electrodeposited chitosan. LVSED, Calibration bar: 10µm.*

Thin HAp coating layer.

The same analysis described before was applied for the chitosan layer applied by electrodeposition on the thin HAp coating. In Figure 18 the surface difference can be clearly noticed. The surface aspect is smoother and some pores can be found in the surface, product of the electrodeposition process as mentioned before.

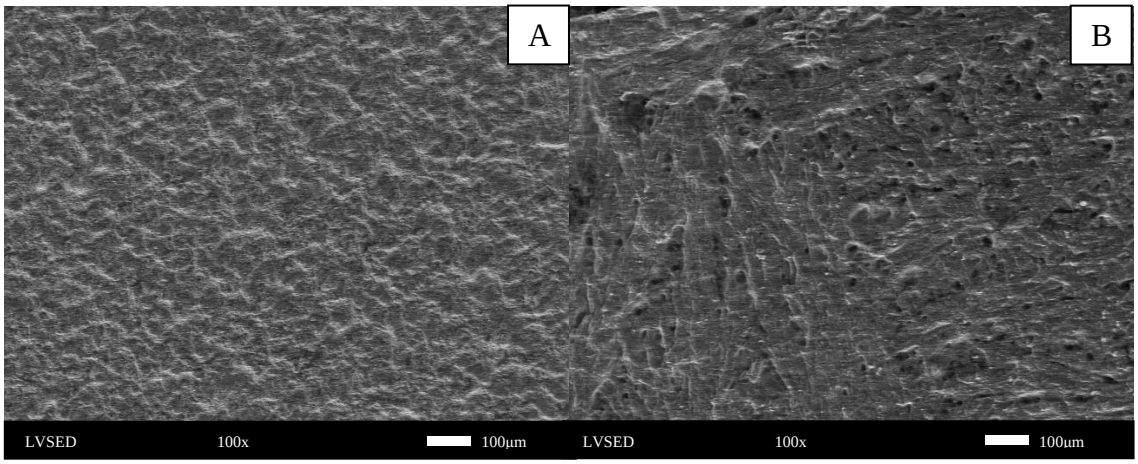

Figure 18 *Surface analysis with a magnification of 100x for A) thin HAp coating B) electrodeposited chitosan. LVSED, Calibration bar: 100µm*

At a greater magnification of the chitosan surface, some cracks can be found in the coating. These cracks must be avoided since they could cause delamination, thus the coating will not work properly. Also, in Figure 19 at 500x, it can be seen that certain areas are not completely covered in chitosan. However, it can be concluded that the electrodeposition method works with the extra the HAp layer.

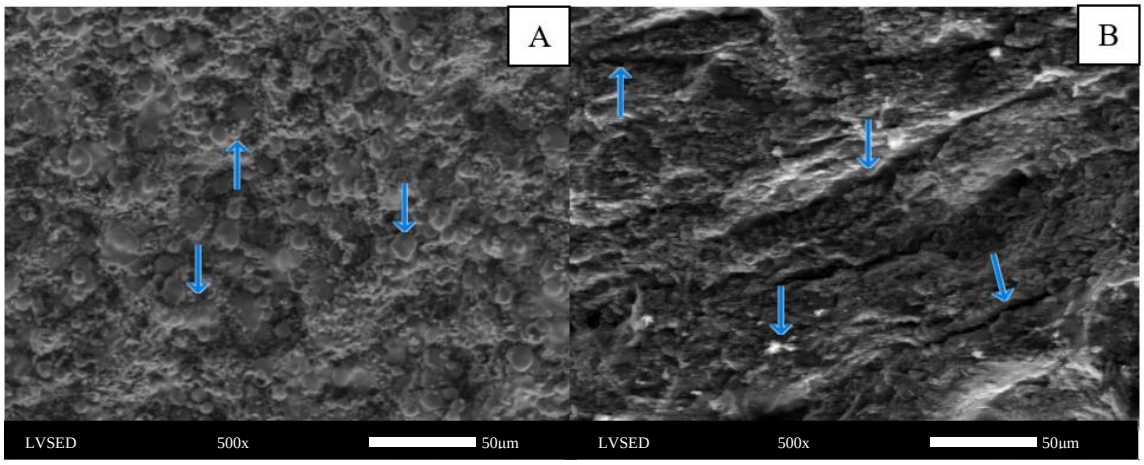

Figure 19 *Surface analysis with a magnification of 500x for A) thin HAp coating B) electrodeposited chitosan. LVSED, Calibration bar: 50µm*

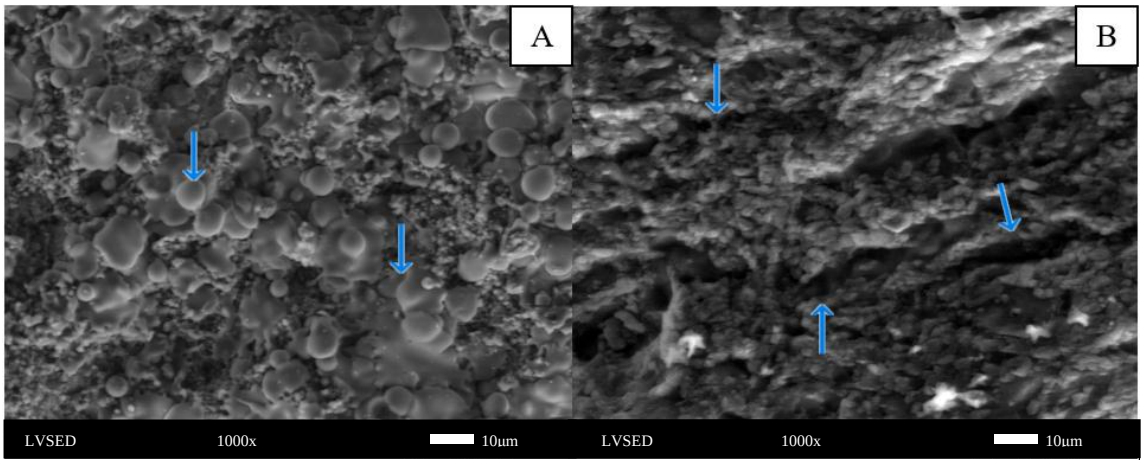

Figure 20 *Surface analysis with a magnification of 1000x for A) thin HAp coating B) electrodeposited chitosan. LVSED, Calibration bar: 10µm.*

Simple adsorption.

The procedure, as in electrodeposition, started by analyzing the surface of each sample to verify the addition of chitosan. In figures above the surface at different magnifications can be compared for both, thin and thick HAp coating layers with the addition of chitosan by simple adsorption.

Thick HAp coating layer.

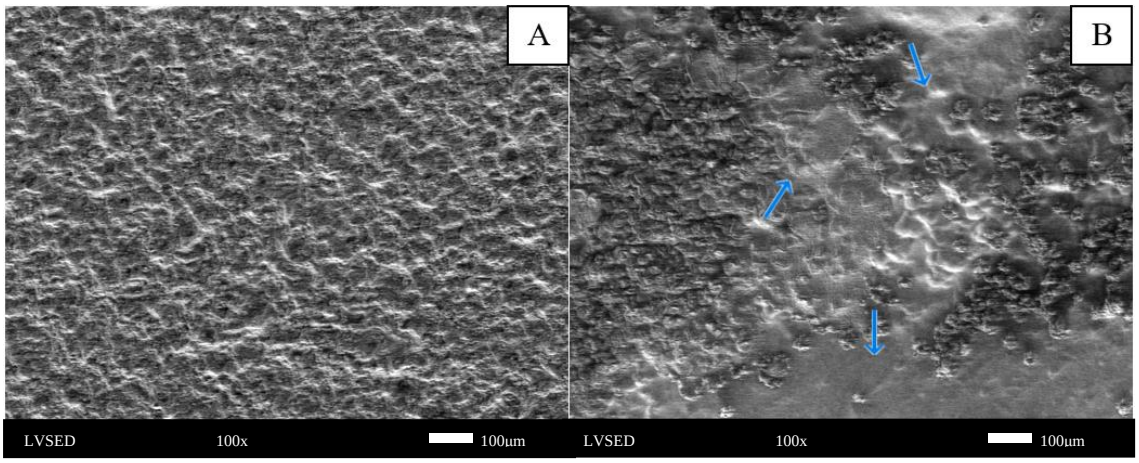

Figure 21 *Surface analysis with a magnification of 100x for A) thick HAp coating B) chitosan by simple adsorption. LVSED, Calibration bar: 100µm.*

In Figure 21 it can be seen that with simple adsorption, the chitosan layer created on top of the HAp coating is more irregular in comparison to the electrodeposition method. The smoother areas shown in this figure represent the concentration of chitosan solution in the surface. However, it is necessary to analyze the uncoated zones to determine if the chitosan solution was absorbed through the HAp pores and cracks.

In certain zones of the smoother chitosan areas, some agglomerations can be found. These agglomerations of chitosan are porous and they can be a product of the filtration of the chitosan solution. The filtration process could not be the appropriate and certain particles can pass through the filter which affect the simple adsorption method, however, the rest of the coating is completely smooth in the surface.

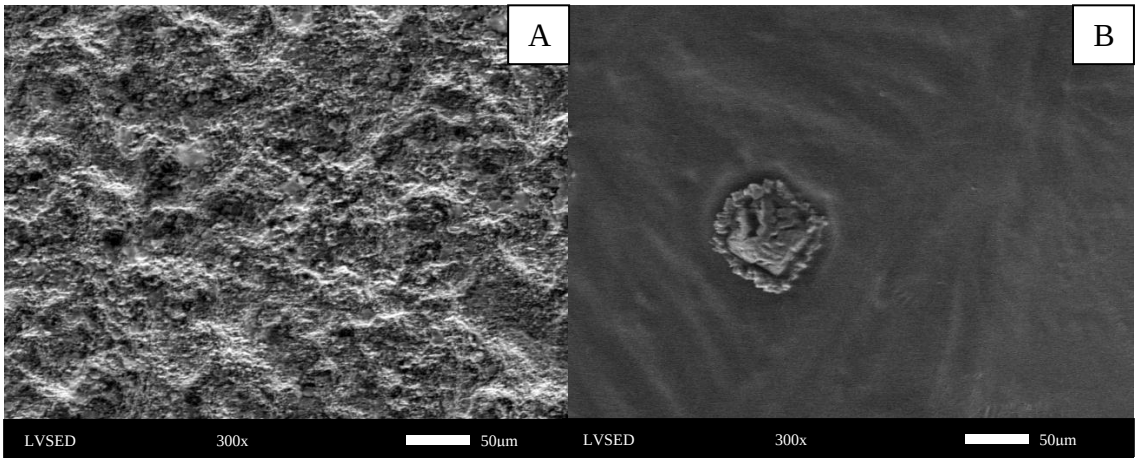

Figure 22 *Surface analysis with a magnification of 300x for A) thick HAp coating B) chitosan by simple adsorption. LVSED, Calibration bar: 50µm.*

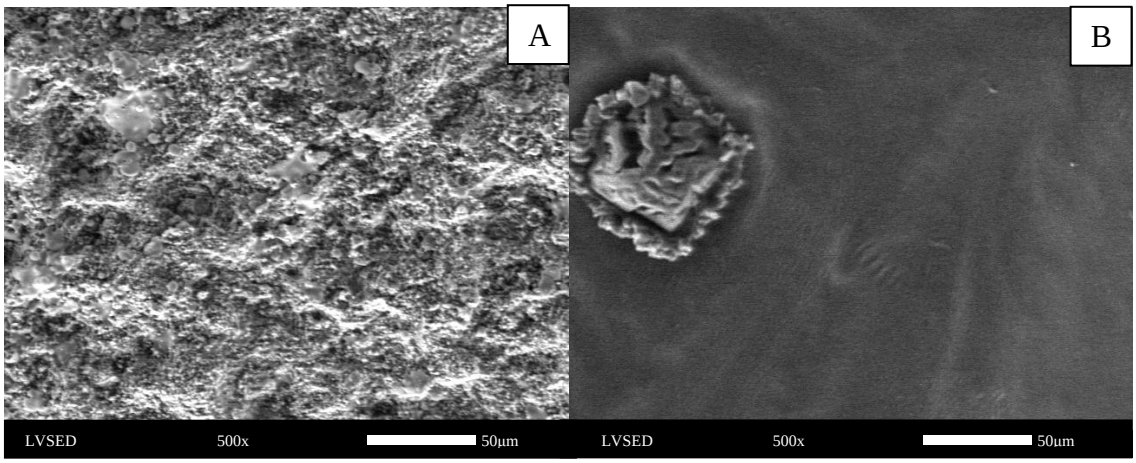

Figure 23 *Surface analysis with a magnification of 500x for A) thick HAp coating B) chitosan by simple adorption. LVSED, Calibration bar: 50µm*

Thin HAp coating layer.

In this section the results obtained for the simple adsorption of chitosan on the thin coat of HAp are presented. The sample analysis in this section was made with the same procedure previously described, which begins with a superficial inspection of the specimens to ensure that there is a chitosan coating layer

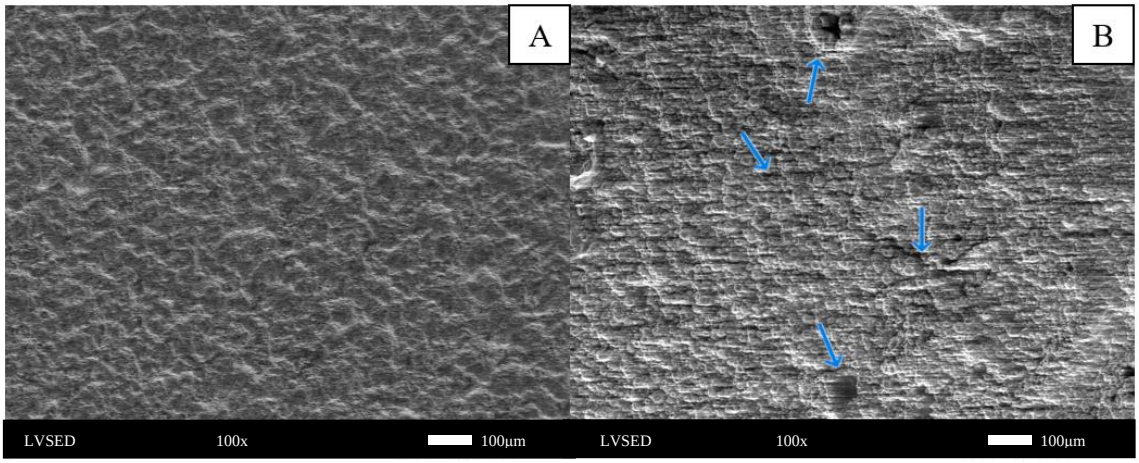

Figure 24 *Surface analysis with a magnification of 100x for A) thin HAp coating B) chitosan by simple adsorption. LVSED, Calibration bar: 100µm*

In Figure 24 the existence of a chitosan layer can be guaranteed as the surface structure of the sample changes. It is also observed that there are pores on this surface, however they are not so abundant compared to the electrodeposition method. Additionally, it can be noted that,

compared to the chitosan simple adsorption method on thick HAp layer, the surface is more rough. For this reason, the possibility arises that chitosan enters more easily into the pores and cracks of the thin HAp coating since there is no agglomeration of the solution on the surface of the coating.

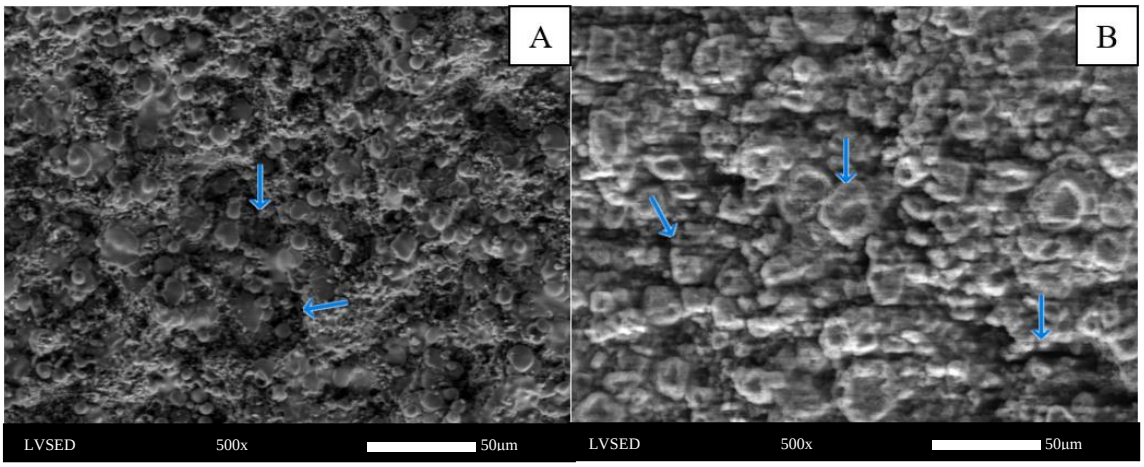

Figure 25 *Surface analysis with a magnification of 500x for A) thin HAp coating B) chitosan by simple adsorption. LVSED, Calibration bar: 50µm*

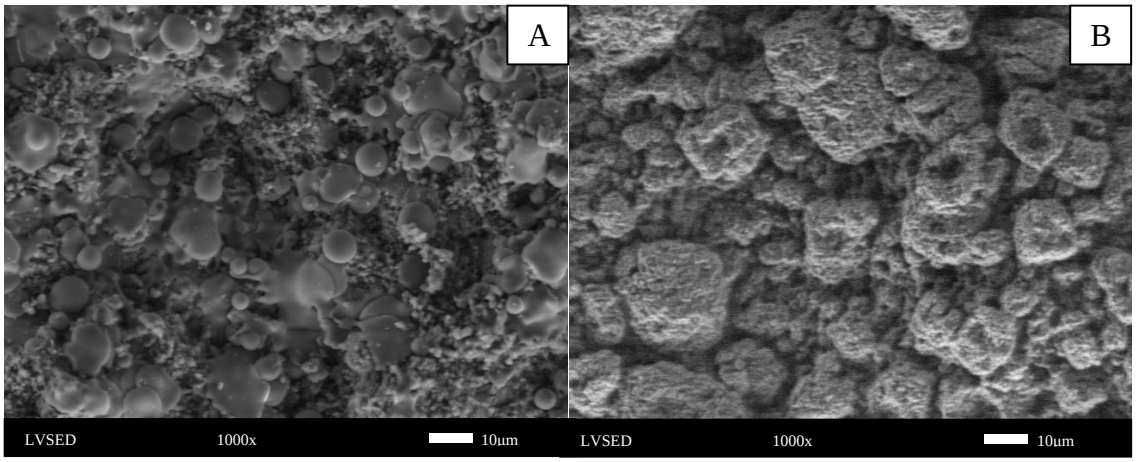

Figure 26 *Surface analysis with a magnification of 1000x for A) thin HAp coating B) chitosan by simple adsorption. LVSED, Calibration bar: 10µm.*

It should be emphasized that for the simple adsorption method, the specimens presented delamination of the chitosan coating, as shown in Figure 27. With this results, it can be verified that the parameters used during this process are not adequate since there is no good adhesion between coating layers.

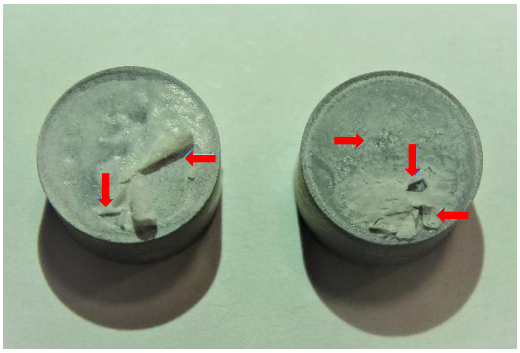

Figure 27 *Chitosan layer delamination*

Budget

This section shows the costs for both materials and machinery used for this project. The following table summarizes these costs.

Table 8 *Total cost for the materials, machinery and equipment for the project*

Material	Description	Cost
Titanium Rod	1 ½ ft. of material with ½ inch of diameter (30 samples with 8mm of height)	$ 79,50
Hydroxyapatite powder	500 gr of synthesized powder	$100
Chitosan	1L of solution	$50
HVOF Machinery	Approx. 3 operation hours	$1000
SEM	$80/Hour (14 samples, 3 hours each)	$3360
Total		$4589,50

It is important to mention that not all the values in the table were applied for this project since certain equipment and materials were granted by Universidad San Francisco de Quito. These include the hydroxyapatite powder and chitosan. Additionally, SEM analyzes were performed within the university's facilities, so their cost was not applied in this project either.

DISCUSION

By comparing the results of this research with previous projects, it can be verified that the procedures applied are favorable for each process used. It begins by verifying the morphology of the hydroxyapatite powder used in the process. It is clearly seen similarities in the shape of the particles in Figure 29. In both studies these HAp particles are amorphous, that is, they do not have a well-defined spherical shape. In Figure 29 the morphology of the powder used by Rincón et al. (2018) in part A is compared with the morphology of the powder used in this research in part B.

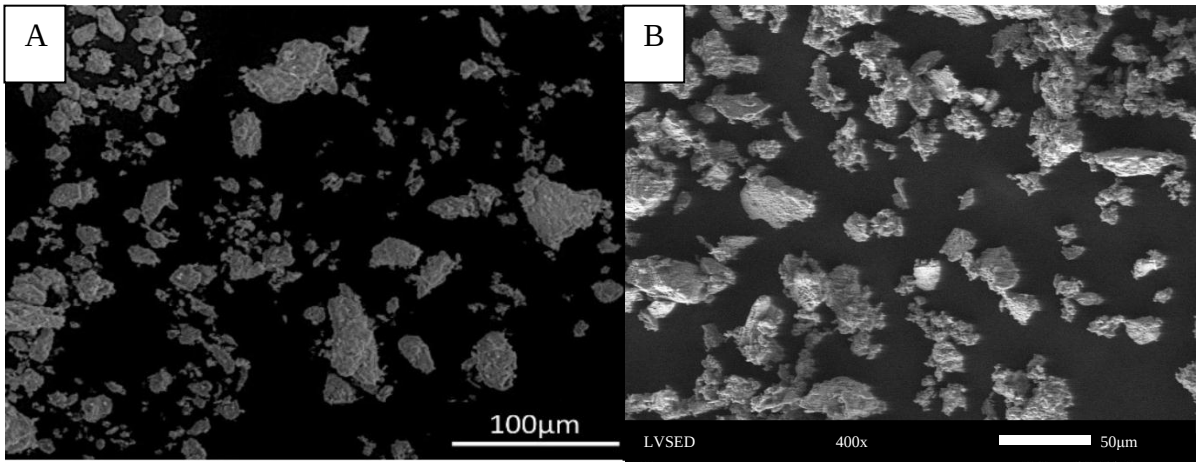

Figure 28 *Morphology comparison for the HAp powder used in A) Rincon et al., B) present investigation*

Also, the parameters for the HVOF process have already been investigated. The main difference in this process is the HVOF gun with which it works and also the machinery used for these investigations. The efficiency of this gun is important to avoid unnecessary waste of the powder and for the process to have a better result.

Additionally, comparing the surface of the HAp coating obtained in this investigation with previous work on this same subject, some similarities can be found, as shown in Figure 29. This figure presents the surface analysis for both studies and it can be noted areas with greater melting of the hydroxyapatite particles, this comparison indicates that the procedure

applied had favorable results even though the parameters and machinery used were modified. Also, it can be noted that the deposition of the coating layer on the titanium exists, so that the parameters can be modified until satisfactory results are achieved.

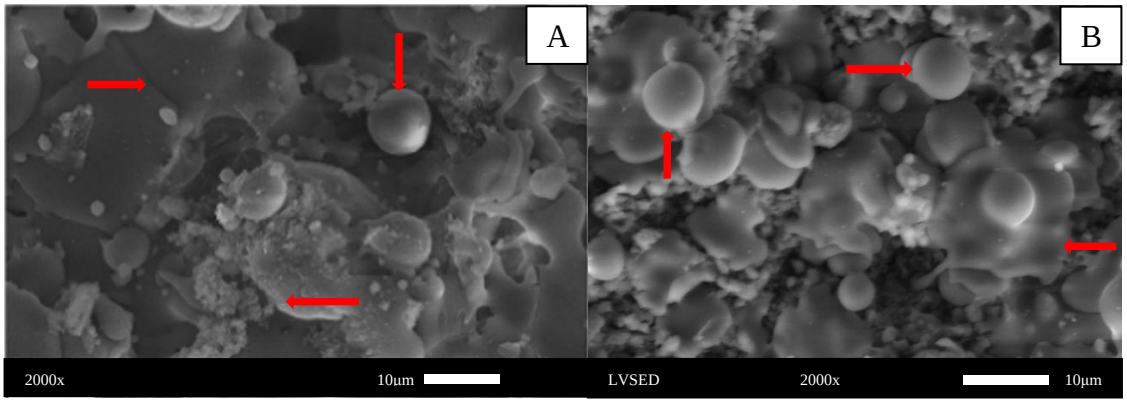

Figure 29 *Superficial comparison for the HAp coating used in A) Harun et al., B) present investigation*

It should also be mentioned that the error that is generated with the procedures used in this investigation for the addition of HAp on the titanium surface is high since these are made manually. The results obtained can be improved and compared with other investigations more precisely if these processes are automated. The automation of the application of the HAp layer also ensures better control of the variables and parameters used. Comparing the results of the thickness of the hydroxyapatite coating with the thickness of the coating of these previous investigations, the ideal total thickness should be approximately 100 µm. However, the maximum thickness obtained in this process is approximately 35 µm as shown in the result analysis section.

On the other hand, the presence of cracks throughout the hydroxyapatite coating on the titanium surface can be noted. As previously mentioned, said cracks benefit the addition of an additional coating layer. In this investigation the extra layer is made of chitosan. This is a viscous solution that can be introduced into the cracks of the hydroxyapatite layer. One of the procedures used for the addition of chitosan is electrodeposition. Previous research is based on

creating a solution of HAp-Chitosan to be electrodeposited on metal surfaces, however, it was found that the use of different techniques for the application of each coating layer is also feasible. As mentioned by Vizcaya-Ruiz et al. (2018), the presence of cracks and pores on the surface of the coating helps the integration between the different layers of the coating, making it stronger and thus also decreases the risk of delamination of the combined coating.

CONCLUSIONS

The HVOF process performed with the Diamond Jet 2600 gun for the application of the hydroxyapatite layer on the surface of the titanium samples had favorable results for the investigation. The parameters used in this process should be slightly modified to obtain the ideal coating thickness. By statistical analysis of the cross section of the HAp coating, it was determined that with 30 passes of the HVOF gun the thickness of the coating is approximately 14μm. On the other hand, with twice as many passes of the gun, the coating layer increases more than double in thickness, with an average of 35 μm. Additionally, because the process was performed manually, the coating layer is not uniform along the sample surface; however, this problem can be reduced with better control of the parameters of speed and distance application of the powder. It is important to emphasize that each component needs preheating to make the process more efficient. The main problem encountered with the application of this coating was the humidity of the powder which caused particle agglomerations which did not flow properly through the equipment. This drawback was reduced by preheating the powder to 150^0C before entering the feeder, which also had a preheating temperature of 100^0C. Additionally, to improve the adhesion of the coating to the titanium surface and to avoid damage to the samples, it was necessary not to exceed 50^0C of initial surface temperature.

This HVOF process can also be improved with the spheroidization of HAp particles. As it can be seen in Figure 6 the morphology of these particles does not resemble a sphere since they are quite amorphous. Since this powder acquires moisture quickly, it is possible that the HVOF process improves in efficiency if these particles are spheres. This morphology would help decrease particle agglomeration and also in fluidity of the powder during the HVOF process.

It was verified with the SEM images that the surface has been completely covered to ensure that the HVOF process has been correctly applied. The images found clearly show the change in the sample surface. On the other hand, with the cross-sectional analysis of this coating, the presence of cracks along the surface can be verified for both the thick and the thin HAp coating. These cracks are favorable for the addition of the chitosan layer since this solution can be introduced into these cracks, thus improving the adhesion between layers of the coating. On the other hand, in the cross section it can also be noted that for the thick coating there are areas with greater fusion of hydroxyapatite particles, while for the thin coating the particles were added one on top of each other. These differences are presented since the process was made manually, and the application velocity of the powder is not constant during the whole process.

Once the analysis of the HAp coating was completed, a chitosan layer was added by two methods, electrodeposition and simple adsorption. The results for chitosan electrodeposition were favorable for both thick and thin HAp coatings. The surface analysis of the samples clearly shows changes in the surface, which is an indicator that there is an adhesion of chitosan on the hydroxyapatite coating. The morphology of the surface for both, the thick and thin HAp coating present some similarities. The presence of pores throughout the surface is important. It can be noticed that these pores are found in greater abundance in samples with thin HAp coating. These pores are the product of the electrodeposition since during the process the bubbles produced can get trapped, thus leaving holes on the coating surface. On the other hand, for the process of simple adsorption, the parameters used must be verified since the specimens presented delamination of the chitosan layer. Additionally, a cross-sectional analysis of the coating should be done to verify which chitosan portion was introduced into the HAp coating. In this analysis, it was noticed some areas in which chitosan particles were agglomerated on the surface. These particles can be a product of the solution filtration process.

This process can be verified to ensure that the solution is free of chitosan agglomerations. Finally, it was verified that it is possible to add an extra layer of chitosan on the HAp coating by different methods, one by electrodeposition and the other by simple adsorption. This research will be required for future investigation that goes into greater detail in each of the parameters used in the different processes.

RECOMMENDATIONS

In order to improve the uniformity of the hydroxyapatite coating it is recommended to automate the deposition process since the error with the manual process is high. With a robotic arm a more uniform coating layer can be obtained along the surface since the application speed and distance parameters can be better controlled. Also, it is important to consider the HAp powder used for the process since the particles are not spheroidal, which increases the particle agglomeration during the process. As a recommendation for future work on this project, an element analysis in the cross section of the coating is essential to quantify the amount of chitosan that entered the HAp layer. With this analysis the results for different samples can be compared. Additionally, with this analysis the best procedure for the addition of the chitosan layer in the coating can be determined. Also, it is important to verify the results obtained in this investigation applying the same parameters to different samples to verify the procedures. On the other hand, since it was verified that the application of chitosan by electrodeposition is possible, it is suggested to vary the process parameters to guarantee a good adhesion between coating layers, thus optimizing the functionality of the coating. The parameters that can be varied in this process are the voltage that is applied to the system and also the time. Additionally, it is suggested to do the same analysis for different concentrations of chitosan solution. One of the disadvantages that is handled with the chitosan solution is its viscosity, however the in-depth analysis of this coating is important. Finally, the simple adsorption method for the application of chitosan should be studied in greater detail. It is recommended to study the process by varying the parameters of time and vacuum pressure as well as the concentration of the chitosan solution. These results can be compared to verify the appropriate parameters.

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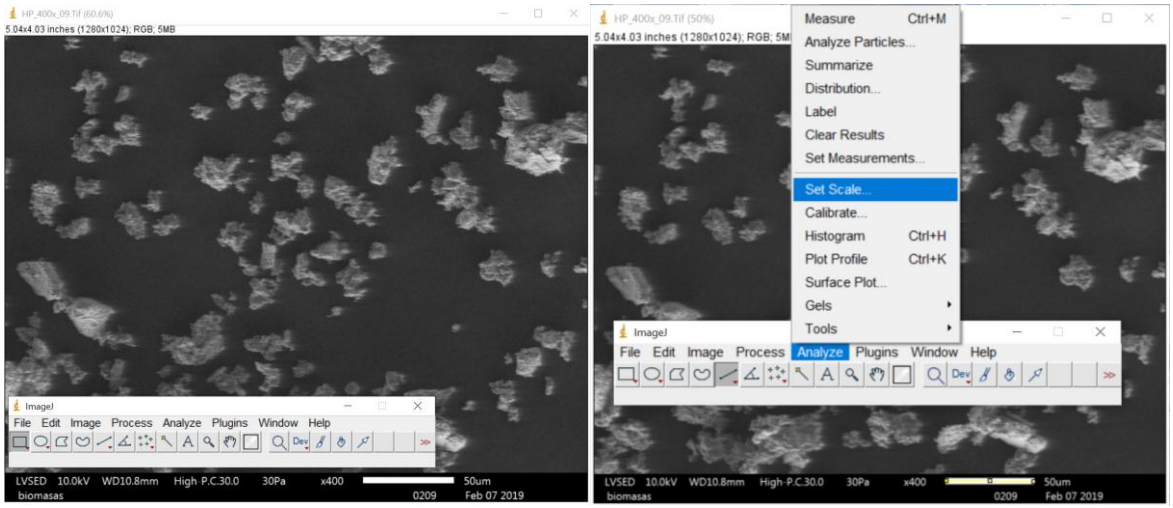
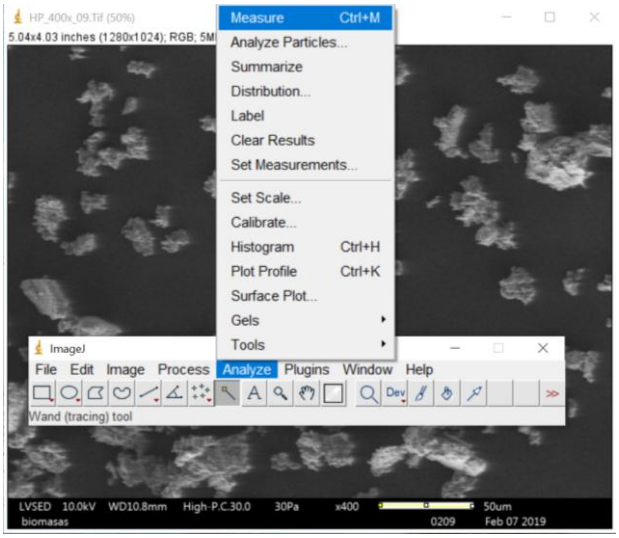
ANNEXES

Annex A: ImageJ configuration





Annex B: Hydroxyapatite composition

Table 9 Heavy elements content of HAp (Rincón et al., 2018)

Element	BHAp (ppm)	CHAp (ppm)	Values accepted ISO 13779-1:2008 (ppm)
As	0.00	0.09	<3
Cd	0.00	0.00	<5
Pb	0.02	0.03	<30
Hg	0.00	0.00	<5