Research, Society and Development, v. 9, n. 4, e27942662, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i4.2662

Tratamento de superfície com ilhas de partículas de prata em Titânio cp: estudo da atividade antimicrobiana

Surface treatment with silver particles isles on Titanium cp: study of antimicrobial activity

Tratamiento superficial con partículas de plata en islas en Titanio cp: estudio de la actividad antimicrobiana

Recebido: 24/01/2020 | Revisado: 05/02/2020 | Aceito: 05/03/2020 | Publicado: 12/03/2020

Patricia Capellato
ORCID: https:// orcid.org/0000-0002-6397-5820
Universidade Federal de Itajubá, Brasil
E-mail: pat_capellato@yahoo.com.br
Cláudia Eliana Bruno Marino
ORCID: https://orcid.org/0000.0003.2660.4577
Universidade Federal do Paraná, Brasil
E-mail: claudiamarino@ufpr.br
Gilbert Silva
ORCID: https://orcid.org/0000-0002-3923-3982
Universidade Federal de Itajubá, Brasil
E-mail: gilbert@unifei.edu.br
Lucas Victor Benjamim Vasconcelos
ORCID: https://orcid.org/0000-0001-5133-2310
Universidade Federal de Itajubá, Brasil
E-mail: vasconcelos.lvb@unifei.edu.br
Rodrigo Perito Cardoso
ORCID: https://orcid.org/0000-0003-0449-0564
Universidade Federal do Paraná, Brasil
E-mail: rodrigo.perito@ufpr.br
Kayam Hamdar
ORCID: https://orcid.org/0000-0003-0286-8498
Universidade Federal do Paraná, Brasil

E-mail: kayam.hamdar@gmail.com

Daniela Sachs

ORCID: https://orcid.org/0000-0002-3767-2455

Universidade Federal de Itajubá, Brasil

E-mail: danisachs@unifei.edu.br

Resumo

Nas últimas décadas, os pesquisadores têm aumentado o interesse no tratamento de superfície com um agente antimicrobiano. Nanopartículas de prata (AgNPs) são amplamente utilizadas em campos biomédicos devido à sua potente atividade antimicrobiana. Assim, neste estudo foram investigadas partículas de prata (ilhas) revestidas na superfície de titânio para aplicação odontológica e ortopédica. O processo de revestimento das partículas de prata na superfície do titânio foi realizado por pulverização, que é uma técnica de deposição assistida por plasma e o titânio sem tratamento foi aplicado como padrão de comparação. Os parâmetros de tratamento com plasma foram otimizados para que o resultado não fosse um filme fino de Ag, mas partículas dispersas de Ag na superfície do Ti-cp. As superfícies das ligas foram investigadas por microscopia eletrônica de varredura (MEV) e espectroscopia de raios X dispersiva em energia (EDS). Para investigar o potencial antibacteriano *Staphylococcus aureus e Escherichia coli* foram utilizados no ensaio de difusão em ágar. Os resultados foram analisados por análise de variância (ANOVA), a fim de verificar diferenças significativas na atividade antimicrobiana entre amostras que não mostraram diferença entre as superfícies estudadas. Para partículas de prata dispersamente depositadas (ilhas) sobre a superfície de titânio para tratamento de 10 minutos, o EDS revelou, ao apresentar aglomerados de prata, que as partículas não foram adequadamente dispersas na superfície, logo a baixa efetividade na atividade antibacteriana.

Palavras-chave: Tratamento de superfície; Atividade antimicrobiana; Implantes dentários; Nanopartículas de prata.

Abstract

During the last decades, researchers have been growing the interest in surface treatment with an antimicrobial agent. Silver nanoparticles (AgNPs) are widely used in biomedical fields due to their potent antimicrobial activity. So, in this study was investigated silver particles (isles) coated on titanium surface for dental and orthopedic application. Silver particles coating process on titanium surface were performed via sputtering that is a plasma-assisted deposition technique with and titanium without treatment was applied as comparing standard. Plasma treatment parameters were optimized so that the result was not a thin film of Ag but dispersed particles of Ag on the Ti-cp surface. The alloy surfaces were investigated using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). In order to investigate antibacterial potential Staphylococcus aureus and Escherichia coli have been used at Agar diffusion assay. The results were analyzed by analysis of variance (ANOVA) in order to verify significant difference antimicrobial activity between samples that have shown no difference between the surfaces studied treatments. For silver deposition scattered particles (isles) over titanium surface for a 10-minute treatment, EDS revealed by silver clusters that the particles were not properly scattered onto surface, hence, the low effectiveness in antibacterial activity.

Keywords: Surface treatment; Antimicrobial activity; Dental Implants; Silver Nanoparticles.

Resumen

Durante las últimas décadas, los investigadores han aumentado el interés en el tratamiento de superficie con un agente antimicrobiano. Las nanopartículas de plata (AgNP) se usan ampliamente en campos biomédicos debido a su potente actividad antimicrobiana. Entonces, en este estudio se investigaron partículas de plata (islas) recubiertas en superficie de titanio para aplicaciones dentales y ortopédicas. El proceso de recubrimiento de partículas de plata en la superficie de titanio se realizó mediante pulverización catódica, que es una técnica de deposición asistida por plasma y se aplicó titanio sin tratamiento como estándar de comparación. Los parámetros de tratamiento con plasma se optimizaron para que el resultado no fuera una película delgada de Ag sino partículas dispersas de Ag en la superficie de Ti-cp. Las superficies de aleación se investigaron mediante microscopía electrónica de barrido (MEB) y espectroscopía de rayos X de dispersión de energía (EDS). Con el fin de investigar el potencial antibacteriano, se han utilizado Staphylococcus aureus y Escherichia coli en el ensayo de difusión en agar. Los resultados se analizaron mediante análisis de varianza (ANOVA) para verificar la diferencia significativa de la actividad antimicrobiana entre las muestras que no mostraron diferencias entre los tratamientos de las superficies estudiadas. Para las partículas de plata dispersamente depositadas (islas) sobre la superficie de titanio para un tratamiento de 10 minutos, EDS reveló al presentar grupos de plata que las partículas no se dispersaron adecuadamente en la superficie, por lo tanto, la baja efectividad en la actividad antibacteriana.

Palabras clave: Tratamiento superficial; Actividad antimicrobiana; Implantes dentales; Nanopartículas de plata.

1. Introduction

Trauma, life expectancy and congenital defects may cause bone and dental loss. This issue causes several problems in a subject's life. These problems described as being since aesthetic to functional, for instance, walking loss and feeding difficulty. Previously, the only way to get over these infortunes was by taking prosthetic aid until implants took place in society. Among advantages of implants the greatest are they cannot be misplaced, neither misused, nor lost. So they bring the user comfort and security (Carlsson, 2014).

Among different bone implants materials, Titanium and its alloys have gained visibility in current years. Researches show biocompatibility between human tissue and titanium. Many alloys with other different compositions, such addicting niobium and tantalum, have been studied due modifying proprieties, making it closer to the replaced material. There are several adaptations that might be performed on material surface as growing nanotubes, adding hybrid films aiming biological interactions (Fernández-Yagüe, et al., 2019)(Arifagaoglu, et al., 2019)(Cruz, et al., 2019)(Gulati, et al., 2016).

Though implants have changed people's life, regards to say that it is implemented by surgical procedure and these procedures bring opportunities to infections. Therefore, different groups of bacteria can infect the region and proliferate through the organism. As consequence, patients linger longer in hospitals, causing more expenses and even risking their own lives by exposing to other contamination sources. In order to avoid this scenario, the use of antibiotics is essential for reducing bacterial infection effectiveness. However, recklessly excessive use of antibiotics has been artificially selecting resistant bacteria. (Pye, et al., 2009).

An auxiliary mean to antibiotics that has been studied is changing material surface, helping to avoid contaminants and antimicrobial function. Silver is a considerable option, once it presents antibiological potential to a wide array of microorganisms. Silver compounds and Ag ions are already applied in procedures as dental work and burn wounds to cease bacterial growth (Kim, et al., 2007). This work has as objective to modify the surface of a standard implant titanium (titanium grade 2) by silver deposition via sputtering. The obtained material will be tested upon two different bacteria groups: Staphylococcus *aureus* (ATCC

6538) and *Escherichia coli* (ATCC 8739). These microorganisms are ordinarily seen in hospital infections and belong to different bacteria groups: Gram-positive and gram-negative respectively.

The proposed modification on titanium surface, being commonly employed in bone and dental implants, is supposed to be able to counter possible opportunistic infections that might occur during the implant surgery and recovery. This would reduce the dependence on antibiotics and grant greater insurance to procedure. As an abridged approach about this study, its purpose is verifying, by agar diffusion test, how silver nanoparticles surface treatment will contend bacterial growth.

As an abridged approach about this study, its purpose is verifying, by agar diffusion test, how silver nanoparticles surface treatment will contend bacterial growth.

2. Matherials and Method

The titanium surface modification was made of silver deposition process in titanium surface was performed via sputtering, an assisted plasma deposition technic. At first, titanium pieces had their surfaces sanded and washed to receive silver coating. Then, physical characterization and antibacterial testes performed to evaluate the material with statistic treatments.

2.1 Titanium sample preparation

The titanium surface modification was made of silver deposition process in titanium surface was performed via sputtering, an assisted plasma deposition technic. At first, titanium pieces had their surfaces sanded and washed to receive silver coating. Then, physical characterization and antibacterial testes performed to evaluate the material with statistic treatments.

2.1.1 Silver cathode preparation

As target in deposition process assisted by plasma, a silver bar Sunshine Minting Inc. 99.9% pure and 55 pounds has been applied. The bar has been prepared by sanding one facet with 200 and 400 sand grain roughness, and then ultrasonicated with isopropyl alcohol and distillated water, for eight minutes each solution.

2.1.2 Silver deposition

In this process, the silver cathode and the titanium sample were places parallel in

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i4.2662

vacuum chamber 1cm apart. Afterwards, locked reactor, the plasma was turned on and its interior cleaned for 5 minutes by generated plasma with hydrogen 50 sccm (standard cubic centimeter per minute) at 0.5 Torr, avoiding contaminants such oils and dust, these could interfere in deposition process. Once cleaning concluded, argon valve has been opened at 50 sccm and the deposition process has started over 0.5 Torr. The whole plasma treatment has been performed with voltage at 700 V, plasma pulse 10 µs and deposition time 10 min. The treatment parameters has been optimized aiming not a thin Ag film, but disperse Ag particles over Ti-cp surface (Surmenev, 2012)(Hirsch, et al., 2019).

2.2 Antimicrobial activity: agar diffusion assay

In this study, the agar diffusion test was the applied method to evaluate the antimicrobial essay in two-group treatment, A, being Ti-cp without superficial treatment, and B, being titanium silver deposited treated. Plus, two different microorganisms, *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8739). The bacteria growing inhibition halo indicates the antibacterial activity magnitude. Assays were performed in triplicate. Therefore, selected bacteria strains have been taken storage culture dish and suspended in sterile saline (0.9% sodium chloride, NaCl). Stock solutions of each bacteria were standardized in 108 cells ml^{-1} using McFarland standards n° 0.5 and spread in agar Muller-Hinton using sterile swabs, then incubated for 24 h at 37°C (Zhang, et al., 2013) (Høiby, et al., 2019).

After incubation, the presented halo diameter around the discs have been measured according Clinical and Laboratory Standards Institute - CLSI (previous NCCLS) to disc-diffusion sensibility assay. The results were analyzed by analysis of variance (ANOVA) in order to verify significant difference antimicrobial activity between samples. To significance, it was performed the Tukey test. The values were expressed by average ± standard deviation with n=3 and significance level $p < 0.05$.

2.3 Superficial characterization

Scan electronic microscopy (MEV) and eye observation have been applied to morphologically characterize the samples surface post 10-minute plasma treatment, element analyzes as well by energy dispersive X-ray spectroscopy (EDS). MEV-EDS equipment from TESCAN, VEGA 3 LMU model (CME – UFPR).

3 Results and discussion

Before covering the surface, a polished sample was analyzed through SEM in order to verify the sputtering process effectiveness. Later, it was also analyzed the material with surface treatment to investigate the process and characterize the material. These micrographs of uncovered and covered surfaces are seen on Figure 1 and Figure 2 respectively. Once in first micrograph is possible to observe some dark spots. They were also identified on second micrograph, so then, they were characterized by Energy-dispersive X-ray spectroscopy (EDS) as being possibly remnants from sanding process with silicon carbide (SiC) considering the high concentration of silicon, presented.

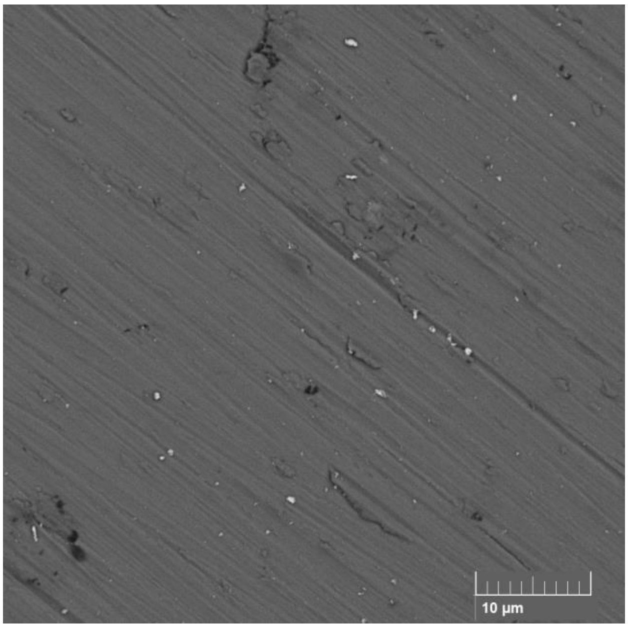

Figure1. Titanium sample surface post sanding.

Still, EDS spectroscopy, Figure 2, were applied to observe silver deposition in three different points, pointed in Figure1. The results point that deposition happened punctually, in other words, deposition has not equally covered the titanium sample surface. It is visible a variation that goes from 7 to 25% in area of Silver (Ag) over surface.

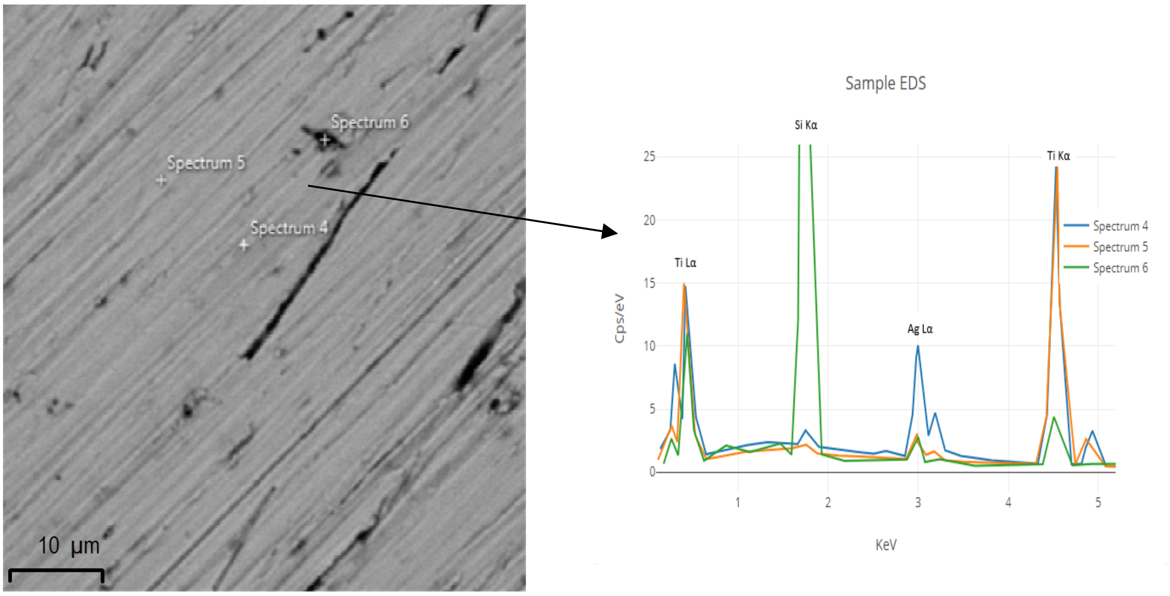

Figure 2. Micrograph of Titanium surface modified by silver deposition via sputtering, and EDS from identified points (spectrum 4, 5 and 6)

Though microscopy, it is also visible a dark bulk on sample surface (spectrum 6). The spectroscopy is able to characterize the bulk as being Silicon particles. It was identified 71.9%weight silicon in this spot (such peak could not fit within graphic), justified by being sanding process remnants, once the sand was made of silicon carbide.

The Petri dishes were prepared with agar Muller-Hinton and the bacteria culture were grown on them. Two comparing groups divided the same dish identified by group A and B, as it is observed in Figure 3.

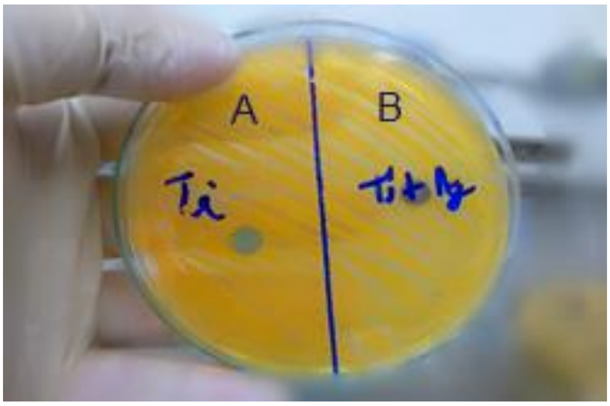

Figure 3. Comparing antibacterial activity in two groups. A representing Titanium sample without silver deposition and B, with silver deposition.

The agar diffusion assay was performed in triplicate to each bacteria strain (*Staphylococcus aureus* (ATCC 6538) and Escherichia coli (ATCC 8739) Figure 4 and the statistic treatment presented no difference between treatment A and B for neither tested bacteria. The inhibition occurred only where the disks were placed. The expected inhibition halo could not be observed.

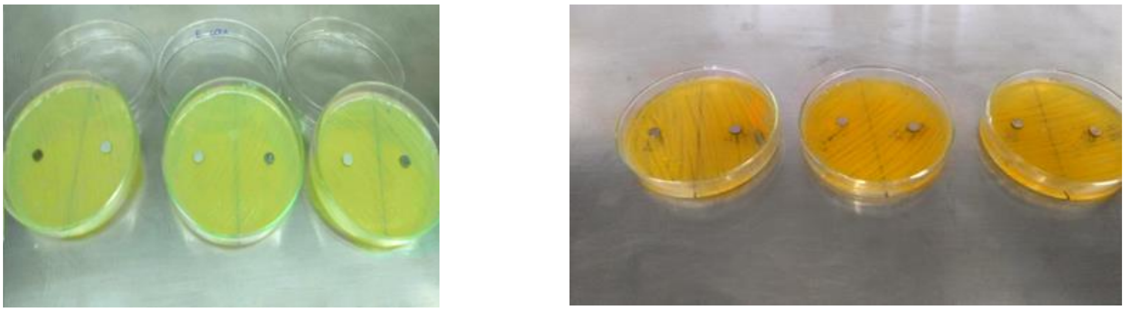

Figure 4. Petri dishes after 24-hour incubation. *Escherichia coli* leftward and *Staphylococcus aureus* rightward. No inhibition halo has been formed in assay, and then no significant differences were observed neither experiments.

In order to cover all sample surface, it was taken sputtering technique due its covering characteristics. It presents simple preparing procedures and can be easily applied over large areas. All benefits justify why it has been widely applied in literature. It consists in evaporating solid materials to plasma and depositing it, onto surface, growing a shallow film (Naoi, et al., 2004)(Okumu, et al., 2005). In addition, the deposition of nanoparticles allows spreading over the surface punctual particles (Okazaki, et al., 2008). Those are forms of reaching different covering.

According the objective of this work, silver nanoparticles were randomly placed over the sample. However, different concentrations were spotted over the surface. Arnell and Kelly(Arnell & Kelly, 1999) have discussed of insulation influence over coating deposition process. Highly insulated materials can cause discharges. These drive droplets ejection of deposition material over target causing film defects. Figure2 allows an overview of this effect by seeing how silver is presented in surface. Also, the same disposition patter was observed by Javadi et al. They performed the deposition (a solution of titanium salt, carrier polymer, polyvinylpyrrolidone) on four specimens, and it is visible through micrograph, cluster formations (Javadi, et al., 2019).

Silver has been considerably applied in countering biofilm formation. It presents a wide antibacterial spectrum, important propriety to avoid different bacteria strains, besides low propensity of bacterial resistance. Implant materials covered by silver have been already being studied and considered promisors due these characteristics (Clupper & Hench, 2001)(Bellantone, et al., 2002).

The silver action pathway is yet unclear, however Ag nanoparticle antimicrobial potential is attributed by presenting a great oxidative potential. Able to, once in direct contact, diffuse throughout cell membrane, whether gram-positive or gram-negative. There are many different considerably know action mechanisms, such as, reactive oxygen species (ROS), DNA damage, and cell membrane rupture. Whenever nanoparticles adhere onto bacteria surface, they alter its natural structure causing interactions with electron donor portion of protein structure. These interactions can enable membrane-bound enzymes and other proteins. Once in cytoplasm, oxidation generates ROS, inducing protein denaturation and DNA damage and then changing cell permeability leading it to death (Pareek, et al., 2018)(Durán et al., 2016).

Li et al. (Li, et al., 2019) has also performed superficial modifications in titanium, one of them, applying silver nanoparticles. In their tests, it is noticeable that ions Ag^{+} were released from titanium surface. The nanoparticles in this study have might not been so available to diffusion as theirs, justifying then, why it have not occurred inhibition halo in agar diffusion test.

Abdel-Fatah et al. also presented a negative result applying silver nanoparticles in order to inhibit bacterial growth (Abdel-Fatah, et al., 2016). The agar diffusion assay was also applied to verify the antimicrobial potential of silver, presenting no inhibition halo. The incapability of silver diffusing through agar bulk might be the source of the obtained result of both studies. Even, comparing results obtained by Shimabukuro et al (Shimabukuro et al., 2019), the low Ag concentration over the titanium sample is also a reasonable cause for the absence of inhibition on agar diffusion assay, whereas it is know the silver nanoparticle antimicrobial potential.

4 Conclusion

For Silver deposition scattered particles (isles) over titanium surface for 10-minute treatment, EDS revealed through presenting silver clusters that the particles were not properly scattered onto surface, in other words, silver deposition presented abnormal uniformity, hence, the low effectiveness in antibacterial activity. The activity can also be justified by concentration of silver and low mobility through agar dish, once silver antimicrobial potential is well known, thus, studies of different concentrations should be performed and then evaluated by methods that allow silver diffusion.

It has been stated that the agar diffusion test is not able to allow silver particles to diffuse throughout agar, in passive means. This limitation requires different techniques to verify the treatment efficiency, as authors point further study to test the material with bacterial biofilms, concerning to achieve better results in antimicrobial activity. This test will favor silver low mobility.

References

Abdel-Fatah, W. I., Gobara, M. M., Mustafa, S. F. M., Ali, G. W., & Guirguis, O. W. (2016). Role of silver nanoparticles in imparting antimicrobial activity of titanium dioxide. *Materials Letters*, *179*, 190–193.

Arifagaoglu, O., Oncul, S., Ercan, A., Olcay, O., & Ersu, B. (2019). *HGF-1 Proliferation on Titanium Dental Implants Treated with Laser Melting Technology*.

Arnell, R. D., & Kelly, P. J. (1999). Recent advances in magnetron sputtering," Surface and Coatings Technology. *Surface and Coatings Technology*, *112*, 170–176.

Bellantone, M., Williams, H. D., & Hench, L. L. (2002). Broad-Spectrum Bactericidal Activity of Ag 2 O-Doped Bioactive Glass. *ANTIMICROBIAL AGENTS AND CHEMOTHERAPY*, *46*(6), 1940–1945.

Carlsson, G. E. (2014). Implant and root supported overdentures - a literature review and some data on bone loss in edentulous jaws. *The Journal of Advanced Prosthodontics*, *6*(4), 245.

Clupper, D. C., & Hench, L. L. (2001). Bioactive response of Ag-doped tape cast Bioglass® 45S5 following heat treatment. *Journal of Materials Science: Materials in Medicine*, *12*(10–12), 917–921.

Cruz, M. A. E., Zanatta, M. B. T., da Veiga, M. A. M. S., Ciancaglini, P., & Ramos, A. P.

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i4.2662

(2019). Lipid-mediated growth of SrCO 3 /CaCO 3 hybrid films as bioactive coatings for Ti surfaces. *Materials Science and Engineering C*, *99*(July 2018), 762–769.

Durán, N., Durán, M., de Jesus, M. B., Seabra, A. B., Fávaro, W. J., & Nakazato, G. (2016). Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. *Nanomedicine: Nanotechnology, Biology and Medicine*, *12*(3), 789–799.

Fernández-Yagüe, M., Antoñanzas, R. P., Roa, J. J., Biggs, M., Gil, F. J., & Pegueroles, M. (2019). Enhanced osteoconductivity on electrically charged titanium implants treated by physicochemical surface modifications methods. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *18*, 1–10.

Gulati, K., Maher, S., Findlay, D. M., & Losic, D. (2016). Titania nanotubes for orchestrating osteogenesis at the bone–implant interface. *Nanomedicine*, *11*(14), 1847–1864.

Hirsch, U. M., Teuscher, N., Rühl, M., & Heilmann, A. (2019). Plasma-enhanced magnetron sputtering of silver nanoparticles on reverse osmosis membranes for improved antifouling properties. *Surfaces and Interfaces*, *16*, 1–7.

Høiby, N., Henneberg, K.-Å., Wang, H., Stavnsbjerg, C., Bjarnsholt, T., Ciofu, O., … Sams, T. (2019). Formation of Pseudomonas aeruginosa inhibition zone during tobramycin disk diffusion is due to transition from planktonic to biofilm mode of growth. *International Journal of Antimicrobial Agents*, *53*(5), 564–573.

Javadi, A., Solouk, A., Haghbin Nazarpak, M., & Bagheri, F. (2019). Surface engineering of titanium-based implants using electrospraying and dip coating methods. *Materials Science and Engineering: C*, *99*, 620–630.

Kim, J. S., Kuk, E., Yu, K. N., Kim, J. H., Park, S. J., Lee, H. J., … Cho, M. H. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology, and*

Medicine, *3*(1), 95–101.

Li, W., Yang, Y., Zhang, H., Xu, Z., Zhao, L., Wang, J., Liu, B. (2019). Improvements on biological and antimicrobial properties of titanium modified by AgNPs-loaded chitosan-heparin polyelectrolyte multilayers. *Journal of Materials Science: Materials in Medicine*, *30*(5), 52.

Naoi, K., Ohko, Y., & Tatsuma, T. (2004). TiO2 Films Loaded with Silver Nanoparticles: Control of Multicolor Photochromic Behavior. *Journal of the American Chemical Society*, *126*(11), 3664–3668.

Okazaki, K., Kiyama, T., Hirahara, K., Tanaka, N., Kuwabata, S., & Torimoto, T. (2008). Single-step synthesis of gold–silver alloy nanoparticles in ionic liquids by a sputter deposition technique. *Chem. Commun.*, *0*(6), 691–693.

Okumu, J., Dahmen, C., Sprafke, A. N., Luysberg, M., Von Plessen, G., & Wuttig, M. (2005). Photochromic silver nanoparticles fabricated by sputter deposition. *J. Appl. Phys*, *97*, 94305.

Pareek, V., Gupta, R., & Panwar, J. (2018). Do physico-chemical properties of silver nanoparticles decide their interaction with biological media and bactericidal action? A review. *Materials Science and Engineering: C*, *90*, 739–749.

Pye, A. D., Lockhart, D. E. A., Dawson, M. P., Murray, C. A., & Smith, A. J. (2009). A review of dental implants and infection. *Journal of Hospital Infection*, *72*, 104–110.

Shimabukuro, M., Tsutsumi, Y., Yamada, R., Ashida, M., Chen, P., Doi, H., … Hanawa, T. (2019). Investigation of realizing both antibacterial property and osteogenic cell compatibility on titanium surface by simple electrochemical treatment. *ACS Biomaterials Science & Engineering*.

Surmenev, R. A. (2012). A review of plasma-assisted methods for calcium phosphate-based coatings fabrication. *Surface and Coatings Technology*, *206*(8–9), 2035–2056.

Zhang, E., Li, F., Wang, H., Liu, J., Wang, C., Li, M., & Yang, K. (2013). A new antibacterial titanium-copper sintered alloy: Preparation and antibacterial property. *Materials Science and Engineering C*, *33*(7), 4280–4287.

Porcentagem de contribuição de cada autor no manuscrito

Patricia Capellato – 14%

Cláudia Eliana Bruno Marino – 14%

Gilbert Silva – 14%

Lucas Victor Benjamim Vasconcelos – 14%

Rodrigo Perito Cardoso – 14%

Kayam Hamdar – 14%

Daniela Sachs – 14%