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ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES ON BIOFILM – DENTAL IMPLANT MODEL

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Abstract: Bacteria are capable of developing biofilms on various types of surfaces, and the bacterial adhesion process can be altered by the characteristics and micromorphology of these surfaces. This way, the properties of biomaterials can be targeted to inhibit bacterial adhesion and colonization. The use of silver is a promising strategy in an attempt to prevent biofilm formation, given its antimicrobial activity. Therefore, the objective of this study was to evaluate the antimicrobial effect of an experimental biomaterial, based on a photopolymerizable orthodontic adhesive (Orthocem UV Trace), modified by the addition of different concentrations of silver nanoparticles (NAg), on biofilm growth (S. mutans). Initially, the surface roughness of the titanium discs, the gap between implant/component and torque/untorque were evaluated. For the biofilm experiment, titanium discs (5 x 2mm) with treated surface (Ti oxide) were used, on which the experimental material was applied, being: G1: Control – biomaterial without addition of NAg; G2: 50ppm; G3: 100ppm; G4: 150ppm; G5: 200ppm; G6: 250ppm. In the end, 2 specimens/group were selected for SEM. The data were not normal, however they were homoscedastic. Thus, post-hoc Tukey ($p<0.005$) was applied for comparison between groups (Graph 3). The Control group, without the addition of NAg, showed less biofilm growth, while the T200ppm group showed greater growth. The T100 and 150ppm groups were similar to each other, as were the T50 and T250. Considering that the addition of NAg did not present the expected antimicrobial effect and that the reason may have been the unavailability of these on the surface, allowing direct contact with the bacterial biofilm, future research must be conducted, seeking to remedy these difficulties and seeking to highlight the antimicrobial effect of NAg.
Keywords: Silver nanoparticles. Bacterial biofilm. Dental implants. Dental biomaterials. *Streptococcus mutans.*

INTRODUCTION

The oral cavity provides an ideal environment for the formation of highly complex biofilms, as it houses more than 700 species. [1][2][3] Although oral tissues have an efficient defense mechanism for reducing biofilm – epithelial desquamation [4], the vulnerability of biomaterials to bacterial contamination occurs during surgical installation [5] and remains due to its transmucosal placement, as part of the implant structure is exposed to the oral cavity permanently, and there is no effective measure to prevent bacterial attachment to the implanted material. [6] which harbors a plethora of biofilm-forming bacteria. Due to its trans-mucosal placement, part of the implant structure is exposed to oral cavity and there is no effective measure to prevent bacterial attachment to implant materials. Here, we demonstrated that UV treatment of titanium immediately prior to use (photofunctionalization

Bacteria are capable of developing biofilm on various types of surfaces, such as living tissue, dentures and dental implants.[7][8] The formation and composition of the acquired film may vary between surfaces, but it begins with the adhesive film promoted by saliva, which makes bacterial adhesion possible. [9] The accumulation of biofilm can lead to the development of peri-implant diseases, which can lead to implant loss and its complications. [10][11][12][13]

Recently, a total of 12 bacterial phyla, 123 bacterial genera and 351 bacteria were identified from salivary metagenome/metatranscriptomic reading, with the most abundant genus being Streptococcus, which together with Prevotella and Veillonella constitute approximately 70% of all DNA and RNA.[14] The bacterial adhesion process

can be altered by surface characteristics and micromorphology of the implants, as well as by surface energy, roughness and/or chemical properties. This way, the properties of biomaterials can be directed to inhibit bacterial adhesion and colonization.[15][16] [17] The prospects are promising in finding a titanium surface treatment that prevents or reduces bacterial colonization and, at the same time, favors the valuable formation of peri-implant tissues.[18]

Silver (Ag) is presented as a promising strategy in an attempt to prevent biofilm formation, given its antimicrobial activity. [7][19][20] It is capable of damaging the bacterial cell membrane, interfering with ion transport, denaturing proteins, inhibiting cellular respiration and DNA transcription, even at low concentrations. [21][22] It is also necessary to observe its biocompatibility, as in rehabilitation with implants, the adhesion of connective tissues is necessary to ensure adequate bone stability and prevent bacterial penetration. [23] However, some difficulties when adding silver nanoparticles to dental biomaterials are observed, mainly in relation to the agglomeration of nanoparticles and heterogeneity in their distribution. [24] [25]Springer-Verlag Berlin Heidelberg.The antimicrobial impact of biogenic-synthesized silver-based nanoparticles has been the focus of increasing interest. As the antimicrobial activity of nanoparticles is highly dependent on their size and surface, the complete and adequate characterization of the nanoparticle is important. This review discusses the characterization and antimicrobial activity of biogenic synthesized silver nanoparticles and silver chloride nanoparticles. By revising the literature, there is confusion in the characterization of these two silver-based nanoparticles, which consequently affects the conclusion regarding to their antimicrobial activities. This review critically analyzes recent publications on the synthesis of biogenic silver nanoparticles and silver chloride nanoparticles by attempting to correlate the characterization of the nanoparticles with their antimicrobial activity. It was difficult to correlate the size of biogenic nanoparticles with their antimicrobial activity, since different techniques are employed for the characterization. Biogenic synthesized silver-based nanoparticles are not completely characterized, particularly the nature of capped proteins covering the nanomaterials. Moreover, the antimicrobial activity of theses nanoparticles is assayed by using different protocols and strains, which difficult the comparison among the published papers. It is important to select some bacteria as standards, by following international foundations (Pharmaceutical Microbiology Manual

To minimize these difficulties, the synthesis of functionalized silver nanoparticles was proposed, directly in the biomaterial, which presents physicochemical characteristics in order to interact with the components of the dental implant, mainly titanium. From a technological point of view, mastering the process of this biomaterial with NAg could lead to the definition of its potential as a dental biomaterial with antimicrobial effect, with the maintenance of the physical-chemical characteristics of the dental implant component, however with the advantage of the antimicrobial effect provided. by the presence of NAg.

Bacterial colonization at the implant/abutment interface (*gap*), formed in two-part implant systems, is still a major challenge in implantology today [2-26]. The space connecting the implant to its internal cavity can act as a reservoir for pathogenic agents, causing biological problems [3-27]. Bacterial communication at the implant/abutment interface is observed to be the most important factor in the occurrence of inflammatory

reactions around the implant, regardless of the stability, design and engineering of the implant connection. Thus, several studies seek an ideal protocol for disinfection and decontamination of implants after peri-implant diseases, [28–31] but few evaluate the effectiveness of materials to prevent contamination through the implant/abutment gap. Currently, some products are already used for disinfection and sealants (Berutemp 500 T2, Carl-Bechem, and Kiero Seal [polyvinyl siloxane (PVS)], Kuss Dental) and chlorhexidine (Chlorhexamed [CHX] 1% Gel, GlaxoSmithKline).[30,32]

Given these difficulties, the present study proposed a material that presents the possibility of sealing the gap formed by the implant/abutment interface, in an attempt to reduce peri-implant contamination and/or internal contamination of the implant, due to the antimicrobial properties of silver. With this intention of clinical applicability, a prior assessment of the size of the gap formed at the interface was carried out, in addition to measuring the torque/untorque, if any clinical intervention was necessary.

Therefore, the objective of this study was to evaluate the antimicrobial effect of an experimental biomaterial, based on a photopolymerizable orthodontic adhesive (Orthocem UV Trace), modified by the addition of different concentrations of silver nanoparticles (NAg), on biofilm growth.(*S. mutans*).

MATERIAL AND METHOD

Initially, the surface roughness of the titanium discs, with different surface treatments (machined, treated and blasted with titanium oxide), were evaluated to select the most appropriate condition, as the roughness of the implant surface can influence the adhesion of the bacterial biofilm. and, it can also influence the adhesion of the experimental biomaterial to the titanium disc, in the dental implant model. The roughness of the experimental biomaterial with different concentrations of NAg was also evaluated.

The geometry of the implant/component interface affects the risk of bacterial contamination [33–36] and internal implant colonization. This interface provides two types of fitting to receive implant-supported prostheses: external connection (external hexagon) and internal connection (internal hexagon and Morse taper). With the intention of clinical applicability of this experimental biomaterial for sealing the gap formed at the implant/component interface, in an attempt to reduce contamination, the size of this *gap* was previously measured. The torque and distortion between implant/component were measured to verify possible interference from the application of the experimental biomaterial on the gap, which could generate clinical difficulties, in the event of the need for any intervention.

For the biofilm growth test, a photopolymerizable orthodontic adhesive was used (Orthocem UV Trace, Dentscare Ltda, Joinville, Brazil), modified by the addition of different concentrations of NAg (experimental biomaterial), applied to the surface of titanium discs, to evaluation of the antimicrobial effect of silver, through the biofilm growth assay with S. mutans. In the end, two specimens/group were selected and prepared for SEM. The control group was the biomaterial without inclusion of NAg.

SURFACE ROUGHNESS: DENTAL MODEL/IMPLANT AND EXPERIMENTAL BIOMATERIAL

The test specimens were made with Titanium discs (5mm x 2mm) with different surface treatments. The following were evaluated (n=5): Group U (Machined), Group T (Treated) and Group P (Polished). Surface roughness was measured with the aid of

an optical profilometer (3D (ZeCage, Zygo Corportion, Devon-Berwyn, Pennsylvania, USA), presenting a quantitative analysis of roughness. Surface morphology was analyzed by SEM - scanning electron microscopy (JSM 6510 – JEOL Ltd., Tokyo, Japan). Profilometry was performed at the center, 1mm and 2.1mm, from the center, determining the roughness in the Radial (Ra-R) and Tangential (Ra-T) directions, as machining is carried out tangentially. Regarding the surface of the specimens for the biofilm test, surface smoothness was promoted by pressing a glass slide (microscopy) on the surface.

GAP MEASUREMENT IN DIFFERENT IMPLANT MODELS

Three groups (n=5) were evaluated: G= HE: external hexagon, G2= HI: internal hexagon and G3= CM: Morse cone. The components were installed on the implants, according to the manufacturer's recommendation, with a torque of 32N. The cervical region of the implant, where the adaptation between implant/component occurs, was evaluated using SEM. 5 gap measurements were taken on each implant, totaling 25 measurements/ group, with a magnification of 1500x. To check the homoscedasticity of the data, the Levene and Shapiro-Wilk statistical test was applied. To reject the null hypothesis of normality, the Kruskal-Wallis non-parametric statistical test was used to compare the groups. Adopted a=0,05.

TORQUE/UNTORQUE EVALUATION

The Morse Cone implants and their polished and surface treated mini-conical components were divided into 4 groups (n=5): G1P= Polished - Control; G2PR= Polished with orthodontic adhesive (Orthocem UV Trace, Dentscare Ltda, Joinville, Brazil); G3T= Treated - Control and G4TR= Treated with orthodontic adhesive. The implants were fixed in a device ("nut") for the purpose of applying torque/untorsion. A torque meter (Tohnichi) calibrated to apply a torque of 32N/cm (initial reading), as recommended by the manufacturer, and detorque (final reading) was used. The biomaterial was applied to the gap between implant/component and light-cured for 40 seconds, as recommended by the manufacturer. The data were statistically treated using the Student's t-test to compare groups with and without application of orthodontic adhesive for each type of surface treatment (Polished or Treated).

PREPARATION OF SPECIMENS FOR BIOFILM GROWTH

Discs were used with the material that makes up the dental implant (grade 4 titanium – Ti 4) in the shape of discs (5 x 2mm) – dental implant model, using a Ti surface treated with titanium oxide blasting, previously selected by through the evaluation of surface roughness. The experimental biomaterial was applied to the Ti discs (n=6), being: G1: Control – biomaterial without addition of NAg; G2: 50ppm; G3: 100ppm; G4: 150ppm; G5: 200ppm; G6: 250ppm. Then, the samples were packaged and sterilized with ethylene oxide.

ANTIMICROBIAL ASSAY BY BIOFILM GROWTH TECHNIQUE

This technique was adapted (Castilho et al., 2014) and performed in 24-well microplates (Costar, Tewksbury, USA). All procedures were performed under sterile conditions with the following strain of bacteria: Streptococcus mutans ATCC 25175® (American Type Culture Collection –25175®, Microbiologics, St. Cloud, MN, USA). A bacterial suspension with 0.5 McFarland, or 1.5 x 108 CFU/ mL was prepared from a concentration of 10 McFarland, using the Nefelobac scale, in saline. On the 1st day, 1 mL of Müeller-Hinton (MH) (Oxoid® Ltd, London, England)

was supplemented with 5% sucrose and then inoculated with S. mutans. One mL of the suspension was added to each of the 24 wells of the microplate. The microplates were kept in an oven at 37ºC for 24h. After this period, the media + sucrose (1mL/well) on the plates were changed without moving the test discs, which remained for another 24h at 37ºC. After this period, the metallic discs were carefully removed and placed in new 24-well plates with 1 mL of PBS – phosphate-buffered saline, [37] remaining for 1 min on a microplate shaker at low speed, between 2 and 3 rpm., in order to remove dead cells. The titanium disks were carefully removed and placed in another 24-well flat-bottom plates, to which 1mL of MTT [3-(4,5-dimethylthiazol-2-yl bromide)-2,5-diphenyltetrazolium bromide) solution was added.]-(tetrazolium reduction test),[38] at a concentration of 0.5mg/mL of MTT in PBS for each well[38][39][40][41] and incubated at 37ºC for 1 hour.

This cell viability test is based on the absorption of the dye MTT (salt - yellow color) in viable cells and its consequent reduction through mitochondrial activity[42], being converted into formazan (purple color). After 1 h, they were transferred to new 24-well plates and 1 mL of DMSO (dimethyl sulfoxide) was added per well. The plates, wrapped in aluminum foil to block light, were set aside for 20 minutes at room temperature, with slight agitation to solubilize the formazan crystals. Next, 200µL from each well were transferred to 96-well plates to read the absorbance (BioTek, EpochElx800, Sellex Inc., Washington DC, USA / Gen5 (BioTek Instruments Inc., Washington DC, USA) at 570nm. The data were analyzed and treated statistically to verify normality and homoscedasticity. Afterwards, appropriate tests will be applied, such as ANOVA and post-hoc Tukey test ($p<0.005$).

SCANNING ELECTRON MICROSCOPY (SEM) - ENERGY DISPERSIVE X-RAY (EDX)

At the end of the biofilm experiment, 2 specimens/group were processed and metallized for observation by SEM and EDX.

CONTACT ANGLE / SURFACE ENERGY

Before starting the evaluation, the surfaces of the specimens were cleaned with isopropyl alcohol. To measure the contact angle, two liquids were used, one polar (water) and the other non-polar (glycerol), with a drop of each being applied to the surface of the test piece, alternately, with cleaning of the surface between applications. The image of the drop was captured by a digital camera (Nikon D5000 – 105 mm Micro Nikon Lens) and, based on its profile, the contact angle with the surface of the material was traced and measured with the aid of a goniometer.

RESULTS

SURFACE ROUGHNESS: DENTAL MODEL/IMPLANT AND EXPERIMENTAL BIOMATERIAL

The results showed that Ra-T is generally smaller than Ra-R and the center of the samples in GU is deeper, while in GP. The center presents a large variation in depth with entrapment of the material resulting from polishing in the central depression. In the GT, the appearance is uniform, despite having greater roughness, being statistically different from the others= 1.324±0.022 µm, while the GP= 0.156±0.025 µm and the GU= 0.158±0.008 µm, similar to each other.

Based on the results, the treated discs (GT) were selected for the biofilm growth assay, as their results were 10 times higher than the results of the GP and GU Groups, which were similar to each other. Furthermore, it is known

that the surface roughness of implants can influence the adhesion of bacterial biofilm. As for the surface roughness of the specimens with and without NAg (different concentrations), the surface smoothness was promoted by the compression of a glass slide (microscopy) on the surface, therefore, as expected, the surface roughness was negligible. (Figures 1-3).

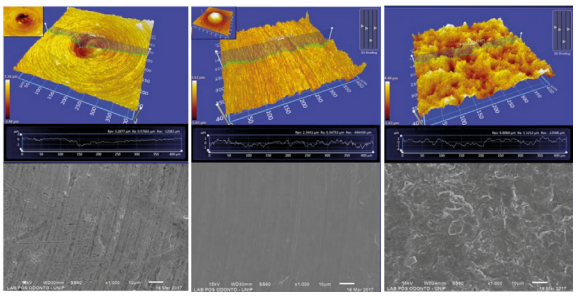
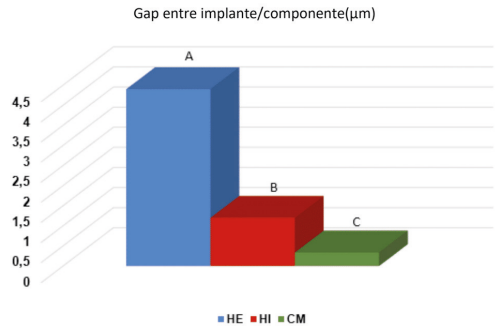


Fig.:1-3: Profiles and SEM of Titanium discs. 1: GU- Machined: Ra= 0.158 (±0.008) µm. The center of the disc is deeper, probably due to the presence of the tool for a longer period of time, which is why it has a higher Ra value than its edges. 2: GP- Polished: Ra= 0.156 (±0.025) µm. Trapping of the polishing material was observed in the central depression (> Ra) previously created by the machining of the disc. 3: GT- Treated: Ra= 1.324 (±0.022) µm. The roughness is greater, but the appearance of the surface is always the same, regardless of the position (center or edge).

GAP MEASUREMENT IN DIFFERENT IMPLANT MODELS

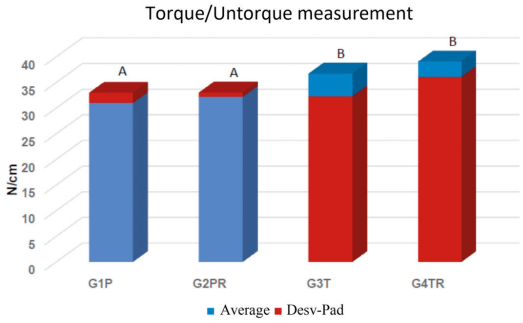
The data did not show normality $p<0.05$. Then the Kruskal-Wallis test was applied. The results demonstrated that there was a significant difference between the groups. The HE group presented the largest gaps, being statistically different from the other groups and the Morse Cone presented the smallest *gap*. (Graphic 1).



Graph 1: Gap (µm) between implant/component of different implant models (HE, HI and Morse Cone. p=0.003. Different letters indicate statistically significant difference - Kruskal-Wallis.

TORQUE/UNTORQUE EVALUATION

The results presented the following values: G1P=31±2, G2PR=32.2±0.84, G3T=32.3±4.44 and G4TR= 36.1±3 N/cm, showing that the groups with the same type of surface treatment were similar to each other, i.e. G1P and G2PR, as well as G3T and G4TR. Results showed that orthodontic adhesive applied to the gap between implant/component did not interfere with untwisting. (Graph 2).

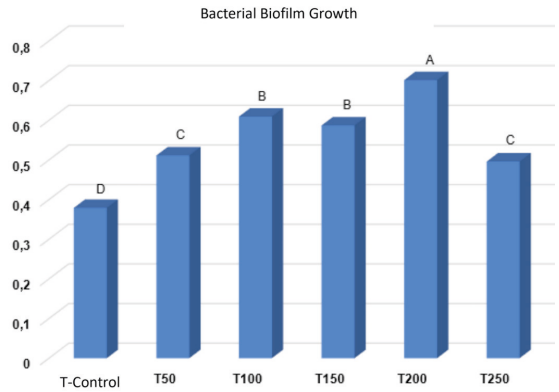


Graph 2: Results showed that there was no difference between the groups with the same type of surface treatment, polished (P) or treated (T).

ANTIMICROBIAL ASSAY BY BIOFILM GROWTH TECHNIQUE

The data were not normal, however, they were homoscedastic. Thus, post-hoc Tukey ($p<0.005$) was applied for comparison between groups (Graph 3). The Control group, without

the addition of NAg, showed less biofilm growth, while the T200ppm showed greater growth. The T100 and 150ppm groups were similar to each other, as were the T50 and T250.



Graph 3: Optical Density Results for bacterial biofilm growth as a function of NAg Concentration from 0 to 250 ppm.

SCANNING ELECTRON MICROSCOPY – SEM/ENERGY DISPERSIVE X-RAY – EDX

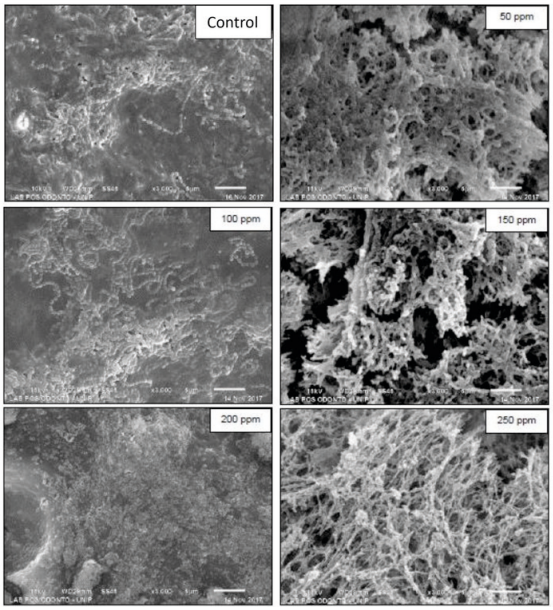


Fig. 4-9: Electron micrographs of the biofilm assay, showing the Control and Groups with different concentrations of NAg, ranging between 50 ppm and 250 ppm. Bar= 5µm.

EDX

	C-K	*O-K*	*Si-K*
50 ppm	48.17	39.6	12.23
100 ppm	48.80	41.4	9.80
150 ppm	65.13	33.11	1.76
200 ppm	51.8	39.89	8.31
250 ppm	64.19	32.76	3.05

Table 1- Chemical elements identified in the EDX reading, demonstrating the absence of NAgs on the surface of the samples.

CONTACT ANGLE / SURFACE ENERGY

The results showed that surface roughness increased the contact angle of titanium discs with a treated surface (T), as they presented an angle of 71o, while the Machined (U) and Polished (P) discs presented an average between 44-48o. As for the specimens with orthodontic adhesive, with and without NAg, they presented similar contact angles to each other, representing greater surface energy in relation to the titanium discs (T). (Table 2).

Treatment	Contact Angle(θ)	
	Polar (deionized water)	Nonpolar (propanetriol = glycerol)
Machined Ti Disc	43	45
Polished Ti Disc	48	48
Treated Ti Disc	71	71
No NAg	49	53
50 ppm	48	53
100 ppm	35	46
150 ppm	50	33
200 ppm	35	35
250 ppm	57	57

Table 2: Contact angles measured for comparison and evaluation of surface energy in relation to surface treatments and NAg concentration.

DISCUSSION

Considering that the T200ppm group showed greater bacterial biofilm growth, the others, T100-T150ppm and T50-T250, were similar to each other, and the Control (without NAg) showed less biofilm growth, it can be said that the addition of NAg did not present the expected antimicrobial effect. Some considerations must be made regarding the evaluations of the properties of the materials used in this study (titanium, orthodontic adhesive with and without NAg), carried out prior to the biofilm test. Regarding the roughness of the titanium discs, it can be observed that the surface treatment with blasting with Titanium oxide (T) increased the surface roughness (10×), while polishing (P) did not reduce it (±1%)., in relation to the machined samples (U). From this experiment, the T discs were selected for the biofilm growth test, precisely because of their greater surface roughness, which would allow better adhesion to the experimental biomaterial with or without NAg.

Furthermore, clinically, it is known that the roughness of the implant surface also promotes greater osseointegration. The surface roughness increased the contact angle of the surface-treated titanium discs (T). Metals normally have high surface energy and, consequently, greater adhesion capacity. [43] However, in the treated discs (T) the opposite occurred, as it is known that the contact angle is inversely proportional to the surface energy, therefore, it can be said that these titanium discs (T) presented lower surface energy in relation to the other discs (U and P) and, therefore, the wetting of its surface will be lower.

Despite this, the contact angle of 71o is still considered partial scattering. On the other hand, the smaller the contact angle, the greater the material's ability to fill surface roughness. [43] However, the viscosity and surface tension of the applied material can influence the filling of these irregularities [43], therefore, the results of measuring the contact angle, which is a simple approach, can be extrapolated clinically, as they allow the knowledge of the wettability of the dental implant surface, predicting cellular behavior on its surface.

On the other hand, the surface roughness of the discs (T) can be beneficial for bacterial adhesion and the osseointegration process, as the rough surface is more hydrophilic than the smooth surface. In relation to the specimens with orthodontic adhesive (with and without NAg), similar to each other in terms of the average contact angle (44-48o), it can be said that they presented higher surface energy in relation to the titanium discs (T).

It is known that surface smoothness makes the material hydrophobic. Therefore, the smoothness of the specimens for the biofilm test was established by compression of an optical microscopy slide, with the aim of making the surface roughness negligible. Despite this, there was bacterial adhesion and biofilm formation, demonstrated by electromyography. In any case, the antimicrobial effect of NAg did not occur as expected. During sample preparation, the surface of the experimental biomaterial was compressed before photopolymerization, which promoted the intrusion of NAg into the polymeric matrix, preventing its availability on the surface, confirmed by EDX. This fact may have contributed to the lack of bacterial inhibition, as NAg acts by contact and the release of ions is minimal and short-range. [25]Springer-Verlag Berlin Heidelberg.The antimicrobial impact of biogenic-synthesized silver-based nanoparticles has been the focus of increasing interest. As the antimicrobial activity of nanoparticles is highly dependent on their size and surface, the complete and adequate characterization of the nanoparticle

is important. This review discusses the characterization and antimicrobial activity of biogenic synthesized silver nanoparticles and silver chloride nanoparticles. By revising the literature, there is confusion in the characterization of these two silver-based nanoparticles, which consequently affects the conclusion regarding to their antimicrobial activities. This review critically analyzes recent publications on the synthesis of biogenic silver nanoparticles and silver chloride nanoparticles by attempting to correlate the characterization of the nanoparticles with their antimicrobial activity. It was difficult to correlate the size of biogenic nanoparticles with their antimicrobial activity, since different techniques are employed for the characterization. Biogenic synthesized silver-based nanoparticles are not completely characterized, particularly the nature of capped proteins covering the nanomaterials. Moreover, the antimicrobial activity of theses nanoparticles is assayed by using different protocols and strains, which difficult the comparison among the published papers. It is important to select some bacteria as standards, by following international foundations (Pharmaceutical Microbiology Manual[40] [44][45][46]

The results of the torque/untorque evaluation showed that the biomaterial applied to the *gap* between implant/component did not interfere with the untorque. Thus, these results are promising, as the initial proposal of this study was to propose a material that presented the possibility of sealing the gap, but that would not interfere with the untwisting, if there was a need for any clinical intervention. Therefore, the size of the gap was also evaluated to verify the feasibility of applying the biomaterial. It is important to highlight that, although the Morse Cone had the smallest gap, between implant/component, with a statistical difference in relation to the other groups (HE and HI), despite its reduced dimensions, the gap allows the passage of bacteria, as these also have micrometric dimensions (between 0.2 and 1.5 μm). This fact is clinically confirmed in peri-implant inflammatory processes. This study intended, through experimental biomaterial with NAg, to present an alternative in an attempt to prevent, mechanically and biologically, the gap/bacteria relationship with the aim of seeking to reduce it to non-pathogenic levels.

Titanium is an important biomaterial and has excellent biocompatibility for the human body. For a long time, only osseointegration was identified as an interfering factor in the success of implants. It is now known that tissue integrity is affected by the surface characteristics of biomaterials, as well as their composition and surface topography. Thus, high surface roughness and energy are favorable to bacterial adhesion, as adhesion and subsequent bacterial colonization were considered key factors in the pathogenesis of biomaterial-centric infections. [47] Therefore, there is a consensus that the adhesion of microorganisms to a surface is a prerequisite for bacterial colonization. Thus, surface topography is critical for the accumulation of biofilms, interfering with the success of rehabilitation treatment with implants. [48]Streptococcus mutans ATCC 25175, Pseudomonas aeruginosa ATCC 27853, and Candida albicans ATCC 10231. The halo zone of inhibition method was performed in triplicate to determine the inhibitory effect of the modified self-curing acrylic resin Dencor Lay-Classico. The surface hardness and compressive strength were examined. The specimens were prepared according to the percentage of beta-AgVO3 (0%-control, 0.5%, 1%, 2.5%, 5%, and 10%

The excellent biocompatibility of titanium surfaces results mainly from their surface properties. Although problems with implant

osseointegration seem to be widely discussed/ resolved, the metabolism of bacteria on these surfaces is still the main reason for the induction of inflammatory processes. [31] Therefore, surface treatment can favor cell adhesion, as well as increase the risk of bacterial infections.

Streptococcus mutans is the bacterium involved in modulating the virulence of bacterial biofilms in the early and late stages of peri-implantitis. [49][50][8]. This was the reason why S. *mutans* was used in the present study.

The antimicrobial properties of silver (Ag), dating back 3,000 years, have their mechanism based on the interaction of Ag with groups of enzymes involved in the metabolism of bacterial cells, leading them to death. [51] With this, NAg were introduced into biomaterials, [52][53] because due to their nanoscale dimensions, they have excellent interaction with microorganisms. [54][55] However, NAg tends to agglomerate when used alone, which hinders its antimicrobial effect, reducing the surface contact area, [24][56] being more efficient when added to a biomaterial [48]. To minimize these difficulties, this study proposed the inclusion of NAg colloidal dispersions directly in the biomaterial used. With the large-scale evolution of nanoscience, silver-based nanostructured antimicrobial properties have been used against microorganisms such as bacteria, viruses and fungi. [57][58]

Biomaterials are undergoing broad nanotechnological development, where dentistry has great expression. In this context, NAg have been shown to be effective antimicrobial components in various materials, such as implants, [20][7] to prevent the formation of biofilms, [44] and for osteogenic induction. [44] Therefore, it is reasonable to believe that, in the near future, NAg will play an important role in oral health.

Most results in dentistry have focused on the antimicrobial efficacy of silver-based systems. Recent studies have demonstrated excellent antimicrobial activity of NAg in materials such as nanocomposites, acrylic resins, adhesives, and implant coatings. NAg has been able to inhibit biofilm without interfering with the properties of biomaterials, and their use as coatings on implants and other materials must be considered. [59][60] [61] However, NAg is not suitable for long-term storage and at high dosage is considered toxic to humans. [62][63]

NAg destabilizes the outer cell membrane and promotes rupture of the plasma membrane of the bacteria S. mutans, and this change can cause bacterial death, reaffirming the mechanisms of nano-antibacterial agents such as silver. [64][65]

Although the antimicrobial mechanism of silver has not yet been completely determined, it is suggested that silver ions denature bacterial proteins and enzymes by binding to reactive groups, causing their inactivation. [66] As in studies involving other compounds, the antimicrobial effect was dose dependent. For S. *mutans*, this decline was clearly evident only at a concentration of 10%. [54][48]Streptococcus mutans ATCC 25175, Pseudomonas aeruginosa ATCC 27853, and Candida albicans ATCC 10231. The halo zone of inhibition method was performed in triplicate to determine the inhibitory effect of the modified self-curing acrylic resin Dencor Lay-Classico. The surface hardness and compressive strength were examined. The specimens were prepared according to the percentage of beta-AgVO3 (0%-control, 0.5%, 1%, 2.5%, 5%, and 10%

The higher the concentration of NAg incorporated into the resins, the greater the antibacterial activity. Previous qualitative analyzes show a reduction in the amount of biofilm as well as its viability with increasing

concentrations. [67]

As reported in the literature, it can be observed that the synthesis of silver-based nanomaterials (NAg and Ag nanocomposites) has become an attractive field of research due to the combination of its biological/ technological impact. Although much progress has already been made in this area, incorrect interpretations and conclusions still occur, as comparison between articles is still complicated, as nanoparticle size measurements vary, as do different strains of microorganisms. [25]Springer-Verlag Berlin Heidelberg.The antimicrobial impact of biogenic-synthesized silver-based nanoparticles has been the focus of increasing interest. As the antimicrobial activity of nanoparticles is highly dependent on their size and surface, the complete and adequate characterization of the nanoparticle is important. This review discusses the characterization and antimicrobial activity of biogenic synthesized silver nanoparticles and silver chloride nanoparticles. By revising the literature, there is confusion in the characterization of these two silver-based nanoparticles, which consequently affects the conclusion regarding to their antimicrobial activities. This review critically analyzes recent publications on the synthesis of biogenic silver nanoparticles and silver chloride nanoparticles by attempting to correlate the characterization of the nanoparticles with their antimicrobial activity. It was difficult to correlate the size of biogenic nanoparticles with their antimicrobial activity, since different techniques are employed for the characterization. Biogenic synthesized silver-based nanoparticles are not completely characterized, particularly the nature of capped proteins covering the nanomaterials. Moreover, the antimicrobial activity of theses nanoparticles is assayed by using different protocols and strains, which difficult the comparison among the published papers. It is important to select some bacteria as standards, by following international foundations (Pharmaceutical Microbiology Manual

CONCLUSION

Considering that the addition of NAg did not present the expected antimicrobial effect and that the reason may have been the unavailability of these on the surface, allowing direct contact with the bacterial biofilm, future research must be conducted, seeking to resolve these difficulties and seeking to highlight the antimicrobial effect of NAg.

GENERAL CONCLUSION

Considering that the addition of NAg did not present the expected antimicrobial effect and that the reason may have been the unavailability of these on the surface, allowing direct contact with the bacterial biofilm, future research must be conducted, seeking to resolve these difficulties and seeking to highlight the antimicrobial effect of NAg.

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