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Antimicrobial Orthodontic Wires Coated with Silver Nanoparticles

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HIGHLIGHTS

- Hydrothermal process was effective used in a one-pot synthesis of silver nanoparticles-based wires.
- Hydrothermal synthesis process did not affect physical chemistry properties of wires.
- Orthodontic wires coated silver nanoparticles from presented antimicrobial activity.
- The material produced is promising for orthodontic treatment.

Abstract: Conventional orthodontic treatment with the use of stainless steel may be detrimental to oral health by promoting demineralizing lesions appearance and increasing adhesion and formation of bacterial biofilm, inducing the development of cavities. An alternative that has been researched to reduce the side effects of orthodontic treatment is the coating of materials with antimicrobial nanoparticles. Nanometric- sized particles increase their surface area and contact with the microbial membrane, consequently intensifying their bacteriostatic and bactericidal effect. In this work, hydrothermal synthesis, a "green" process was used to attach silver nanoparticles (AgNPs) to the surface of two different brands of orthodontic wires. The coated materials were analyzed for their physicochemical properties by scanning electron microscopy (SEM), X-ray diffraction (XRD) and differential scanning calorimetry (DSC), which showed the distribution of AgNPs along the wires without modifying their properties. In the microbiological test, one of the brands showed a statistically significant difference in microbial adhesion and biofilm formation by *Staphylococcus aureus* and

Streptococcus mutans. Results lead to the conclusion that antimicrobial orthodontic wires coated with silver nanoparticles through hydrothermal synthesis is a promising material for the improvement of orthodontic treatment.

Keywords: orthodontic wires; silver nanoparticles; antimicrobial; cavities.

INTRODUCTION

The most common and worrying side effects of treatment with orthodontic appliances are enamel demineralization and periodontal diseases, which appear in approximately 50% of patients under orthodontic therapy. This problem is intensified when fixed orthodontic wires are connected to the teeth through brackets, which prevents access to the dental surface for proper hygiene and thus contributes to bacterial adhesion and plaque formation, increasing the risk of cavities, demineralization and onset of periodontal diseases [1,2].

The use of these devices increases the retention of food particles and causes changes in the oral environment, such as pH decrease, increased colonization by *Streptococcus mutans* and *Lactobacillus acidophilus*, with biofilm formation and plaque buildup, changes that contribute to cavities and decalcification of the enamel, also called white spot lesion, which is unsightly, unhealthy and potentially irreversible [1,3]. The main forming agent of the biofilm responsible for tooth decay progression is *S. mutans* [4]

It is believed that adequate oral hygiene is a fundamental method of prevention that maintains and reestablishes the health status of the gum and periodontal structures during treatment [2]. One of the ways to prevent the development of lesions and cavities is the mechanical removal of bacterial plaques. However, as an additional method of preventing adhesion and microbial colonization, orthodontic movement systems coated with antimicrobial components, such as certain nanoparticles, are being developed [5].

Nanoparticles are materials with remarkable properties that range from 1 to 100 nm, according to the ISO (International Organization for Standardization) [6], and have been widely used as disinfectants in water, hospital environments, as food preservatives and to coat medical devices [7]. When acquiring nanometric size, the materials have their properties altered, such as hardness, active surface area, chemical reactivity and biological activity and, especially in relation to metallic nanoparticles, the antimicrobial activity is increased due to both its reduced size and the high surface ratio on volume [8,9]. This allows for a better interaction with the microbial membrane and increases the biocompatibility of the material, thus increasing the development of new devices and formulations that can be used in the physics, biology, biomedicine and pharmaceutical sectors [9,10,11]. Some metallic nanoparticles, such as titanium (TiO_2), zinc oxide (ZnO) and silver (Ag) are used in the health area due to their antimicrobial activities [5].

Silver nanoparticles (AgNPs), in particular, are well known for microbial inhibition of silver ions (Ag^+), together with the advantages of their nanometric size, which, due to their higher surface area, have an increased antimicrobial activity [8,9]. The antibacterial, antiviral and antifungal activity of AgNPs has been demonstrated in several studies, being effective against the *bacteria S. aureus, Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Listeria innocua* and *Salmonella choleraesuis*, and also against the pathogenic yeast *Candida albicans*, in vitro [11,12,13].

Recently, some methods have been explored for the reduction of silver ions (Ag^+) to nanoparticles (Ag^0). One of these is hydrothermal synthesis, which is considered a "green" method, capable of producing nanoparticles through the control of pressure and temperature [14]. It is one of the most attractive techniques for the processing of nanocomposites, aiding in the process of dispersion and homogeneity of nanoparticles [15].

The mechanism of action exists due to the Ag^+ ions that cause the precipitation of proteins and act directly on the cytoplasmic membrane of the bacterial cell, effecting immediate bactericidal action and residual bacteriostatic action [9]. Ag^+ ions are released from the nanoparticles and react mainly with the sulfur that makes up most of the membrane proteins, affecting cellular permeability and the viability of the bacteria. However, they may also interact with phosphorus fragments of DNA, preventing their replication and expression of proteins and enzymes, as well as inhibiting the respiratory chain and increasing the generation of reactive oxygen species (ROS), causing cell death [16,17].

These nanoparticles are active against various types of bacteria, including multi-resistant strains [17]. As they are easily incorporated into different materials, silver nanoparticles have been used in bandages to prevent infections and promote healing in antimicrobial pharmaceutical formulations, surgical instruments, antimicrobial insoles to prevent odors, water and air purifiers, food coolers to retard deterioration, among other applications. However, the increase in the production and application of nanoparticles has provoked a wide debate about the potential risks to the environment and human health [7,18].

In dentistry, they can act as a form of control of the formation of dental biofilm and demineralization, being explored as a coating of prosthetic devices, topical formulations and as an antibacterial constituent of dental resins [5,10]. Silver particles were also incorporated into composite resins mass to prevented the accumulation of dental biofilm on the surface of the restorative materials given the gradual and constant release of silver ions, and the antimicrobial action on *Streptococcus* spp. [19,20]. NiTi orthodontic braids containing silver had an effect against the adhesion of *Lactobacillus acidophilus*, bacteria responsible for the progression of cavities [1]. Due to this evidence, this study aimed to analyze the antibacterial and physico-chemical biological properties of orthodontic wires coated with silver nanoparticles obtained by hydrothermal synthesis, in order to increase orthodontic treatment favoring oral health.

MATERIAL AND METHODS

Synthesis of silver nanoparticles and coating of orthodontic wires

Stainless steel arches of two different brands, Abzil® and Orthometric®, were used for the experiments as A and B, respectively. These were cut in 1cm segments and agitated in $AgNO_3$ solution at concentrations of 10^{-1}, 10^{-2} and 10^{-3} mol L^{-1}, used as silver precursors. Through a "green process" called hydrothermal synthesis, in an autoclave-like reactor that allows monitoring temperature and pressure, silver ions were reduced to nanoparticles that have their morphology and dispersion controlled during the process.

Physico-chemical characterization of orthodontic wires

Scanning Electron Microscopy (SEM)

The segments of the control and experimental groups of both commercial brands were fixed with carbon tape in metallic support and observed in JEOL model JMF-6700F high resolution scanning electron microscope.

X-ray Diffraction (XRD)

X-ray diffraction is a technique that allows the study of the microstructure of solid materials, determining their crystalline areas and their percentage crystalline fraction. The diffractograms were obtained using a Rigaku RINT2000 diaffractometer with a power of 40Kv, 50Ma. Detector: D / teX, slits with divergence of 0,25 degrees and Soller primary and secondary of 2,5º of divergence. The wavelength used was K1=1,5406Å and k2=1,5444Å I2/I1=0,5. Region 2 was measured as follows: 5 at 80º. The measurement mode was continuous sweep of 4º/min, step width 0,020º.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) analysis was performed to identify the behavior of the material during temperature change, detecting the amount of energy absorbed or released during heating and/or freezing, in relation to an inert material. The DSC curves were obtained in Mettler Toledo DSC 1 STAR with mass of approximately 9mg, aluminum crucible, inert atmosphere, with heating of 10°C min.

Microbiological test of orthodontic wires

Bacterial Adhesion

For the tests, *S. aureus* strains (ATCC 29213) were obtained from the Research Center on Biodiversity and Biotechnology-BIOTEC, at the Federal University of Piauí. Bacterial adhesion in the wires was determined by using the spread plate method. To that end, a 1mL volume of bacterial suspension ($1x10^6$ CFU/mL) in Mueller-Hinton broth was added to wells containing the strands, and the plates incubated at 37°C for 4 hours. After the incubation period, the strands were subjected to an ultrasonic bath at a frequency of 50 Hz for 5 minutes. The solutions collected after the sonication process were diluted and plated in triplicate in TSA medium ("Trypticase Soy Agar"). The petri dishes were incubated at 37 °C, for 24 hours. The number of colonies in the TSA was counted, and the result expressed in Colony Forming Units / mL (CFU / mL).

Biofilm Formation

S. aureus ATCC 29213 and *S. mutans* ATCC 25925 were selected for the biofilm assay in orthodontic wires. The bacterial cells were grown on Mueller-Hinton Agar (MHA, BD™) at 35±2 °C/24 h for *S. aureus*, in aerobic conditions or Trypticase Soy Agar (TSA, BD™) at 35±2 °C/48 h for *S. mutans*, under micro-aerobic atmosphere. After the appropriate incubation time, a standard inoculum was prepared from isolated colonies at bacterial concentration of 1-2 × 10^8 colony-forming units (CFU/mL), according to CLSI guidelines (2015) [21].

For bacterial attachment on orthodontic wires, (Abzil® and Orthometric®) a bacterial inoculum (5×10^5CFU/mL) was made from the aforementioned bacterial suspension in tryptic soy broth culture (TSB, BD™) with carbohydrates (0.5% glucose for *S. aureus* or 2% sucrose for *S. mutans*) and 100µL of inoculated medium was deposited in the microplate wells. Then, the wires were also placed in the wells in contact with the inoculated medium (untreated and treated wires at the dilutions of 10^{-1}, 10^{-2} and 10^{-3}), then the plates were incubated for 24 or 48 hours at 35±2°C. Then, the wires were dried at 60°C for one hour, and the biofilms in the wires were stained with 500µL of 0.1% (w/v) violet crystal at room temperature for 10 minutes and dried at 35±2°C for 2 hours. Biofilm formation was quantified through the solubilization of the violet crystal in 200µL of 30% (w/v) glacial acetic acid. The optical density was determined after reading in the spectrophotometer at 492 nm and is directly proportional to the amount of biofilm formed [22].

For statistical analysis of the data, one-way ANOVA was utilized with the Tukey post-test and optical densities presented as mean ± standard deviation, in the GraphPad Prism® 6.0 software. A $p < 0.05$ was established for statistically significant data.

RESULTS

Physico-chemical characterization of orthodontic wires

After coating orthodontic wires with silver nanoparticles by hydrothermal synthesis, it was not possible to observe changes in wire color or roughness. There were also no changes in the thickness of the threads, showing that the surface treatment did not interfere in the wire aspect.Sample micrographs, shown in Figure 1, confirm orthodontic wires surface coating by AgNPs, clearly evidenced by their brightness, which forms the white dots observed in the images. Most of the nanoparticles covering orthodontic wires presented spherical-like shape and nanometric size, inferior to 20nm.

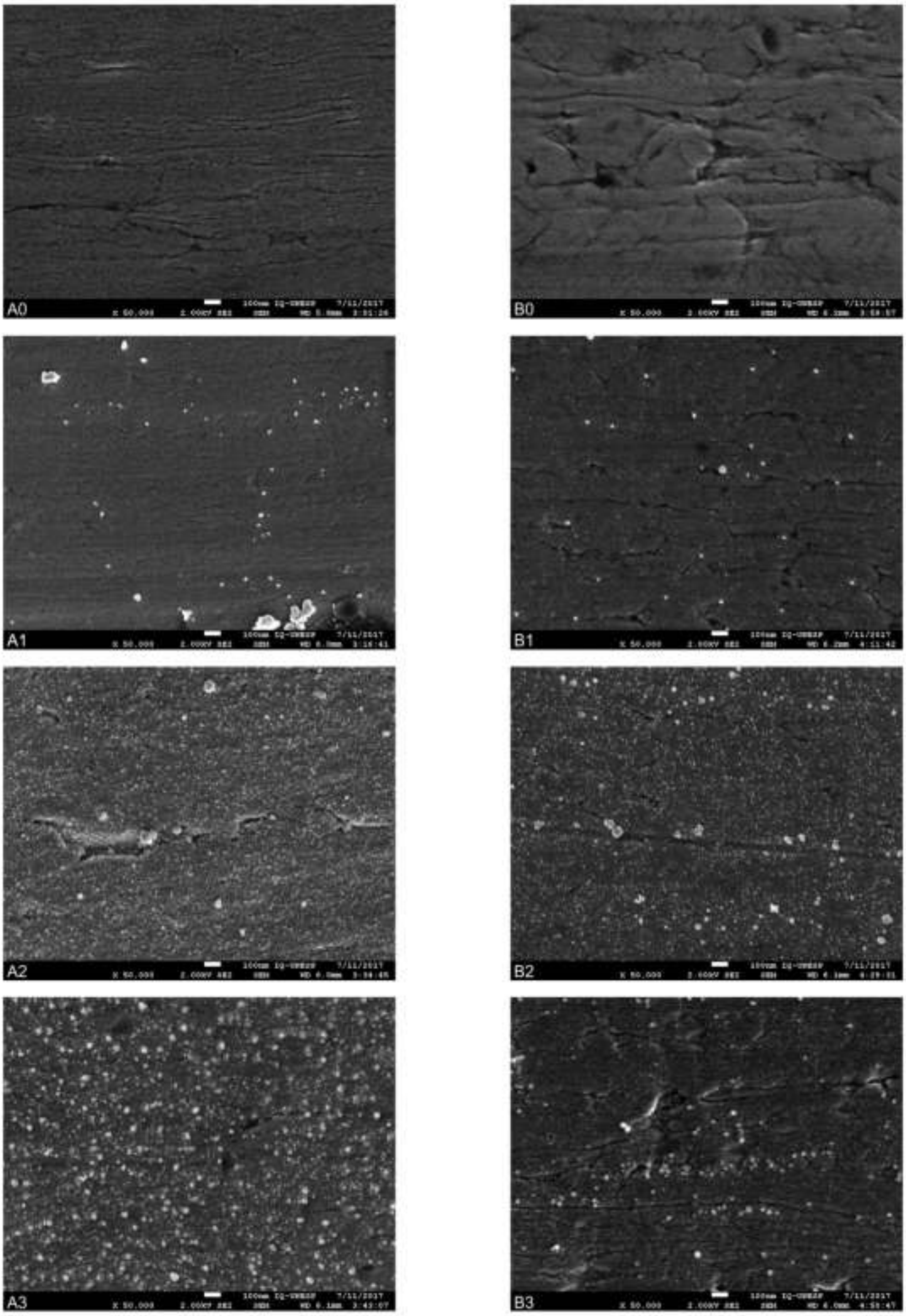

Figure 1. Comparison of the obtained 50,000x micrographs of Abzil® (A) and Orthometric® (B) brands: A0 and B0 are the control segments (uncoated), and A1/B1, A2/B2 and A3/B3 are AgNPs impregnated wires at 10^{-1}, 10^{-2} and 10^{-3} M concentrations, respectively. It is possible to observe greater surface coating of AgNPson the first brand wires, at the lowest concentration tested (A3).

Micrographs show that AgNPs coating occurred in a more dispersed and homogeneous way in Abzil®, which was mostly seen in the tested samples with lowest concentration (10^{-3}M).

Figure 2 shows XRD results, where it was possible to observe two similar diffraction peaks for the samples of the Orthometric® brand,at the 44° and 75º angles, but with a greater intensity at the 44º in B0 (control) and B1; and two smaller peaks at angles 50° and 65°. In these samples, a diffraction peak of 21°

was observed, which was absent in the other groups of the brand (B2 and B3). Abzil® wires also exhibit minor diffraction peaks at angles of 50° and 65°, and 3 similar larger peaks in all groups at 21°, 44° and 75°, and presented a more crystalline structure than the Orthometric® wires.

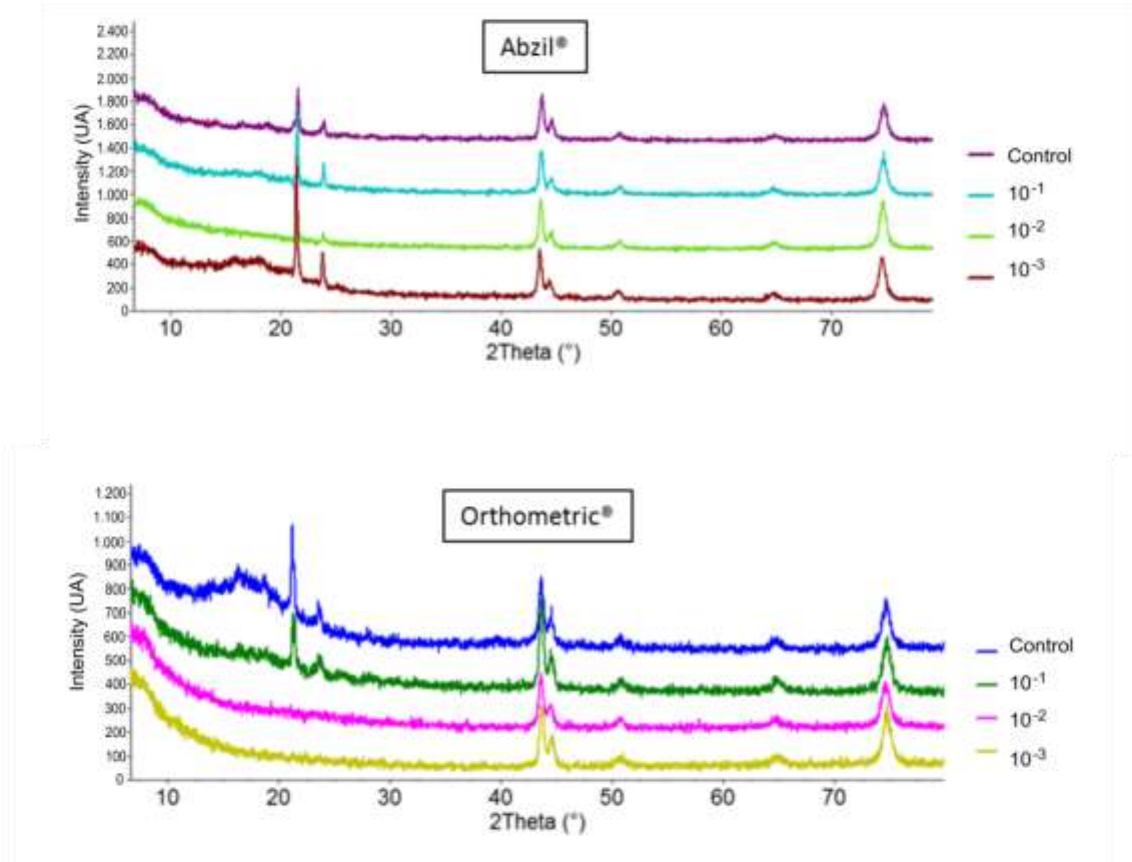

Figure 2. XRD of control and experimental groups of both brands, Abzil® and Orthometric®.

DSC graphic shows a similar curve in both control and AgNPs coated samples for the Abzil® and Orthometric® brands. By comparing DSC of the samples of both brands (Figure 3), two endothermic and one exothermic event is observed, the latter being at -65 °C in groups A0 and A1. The first endothermic event occurred at 60 °C in samples B2 and B3, and the second at 90 °C in sample B3.

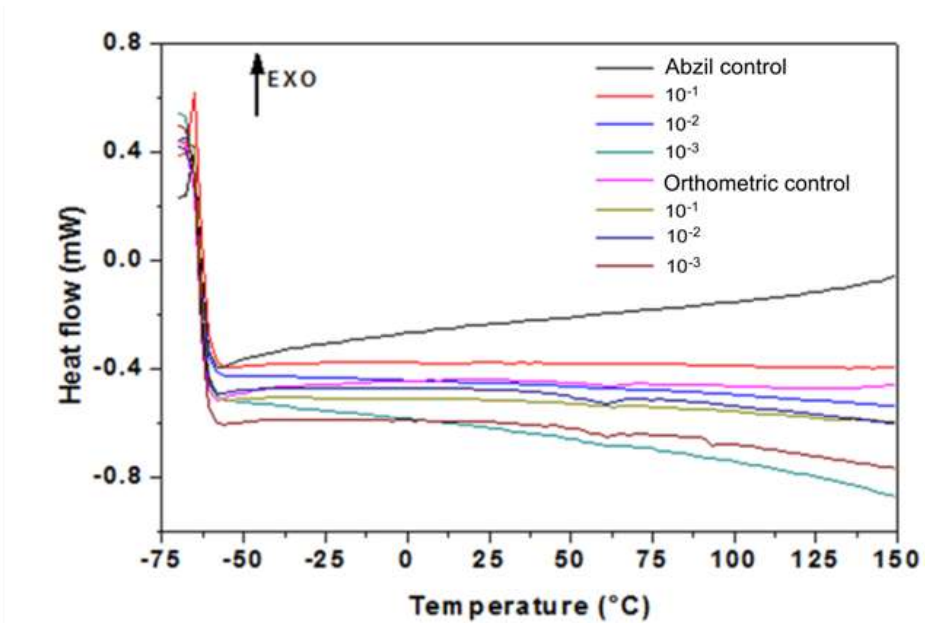

Figure 3. Comparative DSC curves of the 8 groups tested.

Microbiological test of orthodontic wires

Regarding the untreated wires, in the microbiological assay there was a significant difference (p<0,05) in the adhesion of *S. aureus* ATCC 29213 to the wires, with optical density equal to 0.174±0,04 for the Orthometric® wire and 0.113±0,01 for the Abzil® wire, whereas for *S. mutans* ATCC 25925 no significant difference was observed between the amount of bacteria adhered onto the orthodontic wires (0.118±0,007 for Orthometric® and 0.118±0,009 for Abzil®). This result can be observed in Figure 4, where the Orthometric® wires allowed a greater adhesion of *S. aureus*.

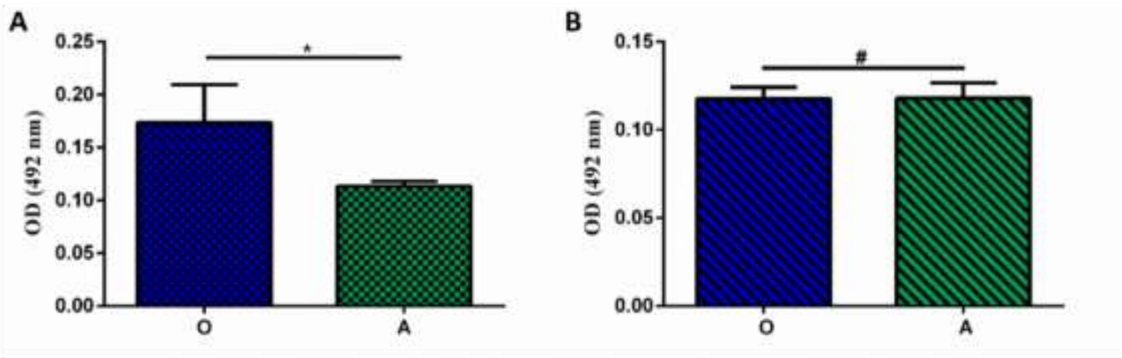

Figure 4. Comparison of bacterial attachment on orthodontic wires. A) Biofilm of *Staphylococcus aureus* ATCC 29213 and B) Biofilm of *Streptococcus mutans* ATCC 25925. * significance (p<0.05); # no significance. O – Orthometric®; A – Abzil®.

In the test performed with treated wires, a significant reduction of bacterial biofilm formation was observed only in the Abzil® orthodontic wires, for *both S. aureus* and *S. mutans*, as it is shown in Figure 5.

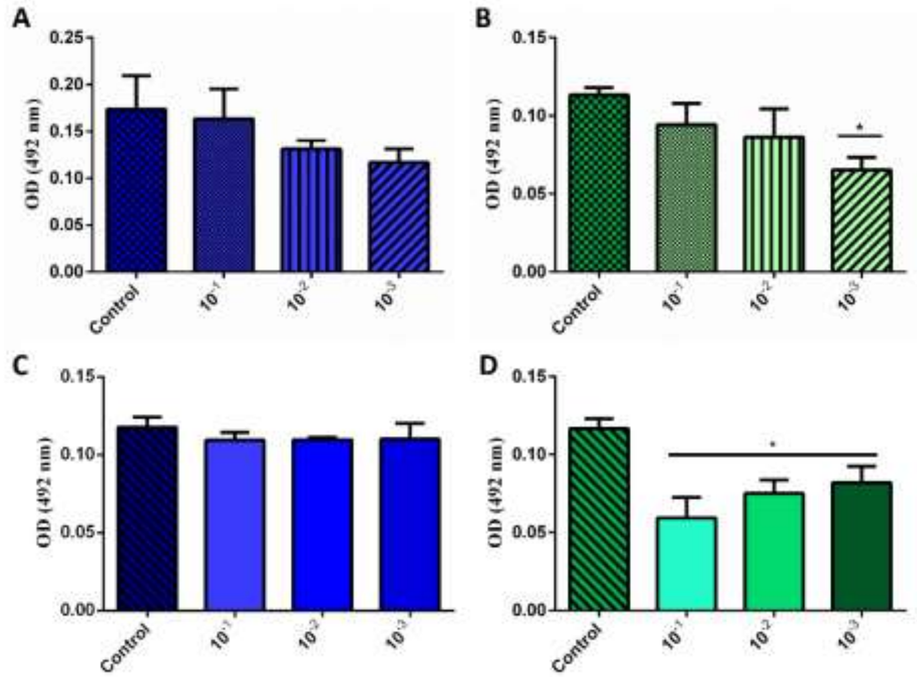

Figure 5. Quantification of bacterial attachment on treated orthodontic wires. The blue bars are for Orthometric® wires and the green bars are for Abzil® wires. A) and B) Biofilm of *Staphylococcus aureus* ATCC 29213; C) and D) Biofilm of *Streptococcus mutans* ATCC 25925. * significance (p<0.05) in relation to control.

Control and treated wires of Orthometric® had no significant effect on adhesion and formation of the bacterial biofilm, whereas those of the Abzil® brand were effective against *S. aureus* and *S. mutans*.

DISCUSSION

According to physical-chemical characterizations, hydrothermal synthesis is an efficient method for silver nanoparticles production and surface coating of stainless-steel orthodontic wires. As well as reported by Oliveira and coauthors [23], macroscopically it was not possible to observe changes in the wire aspect after AgNPs coating.

SEM is an important technique used for the study of nanoparticle morphology and size, as well as allowing for the analysis of sample homogeneity [24] and, according to its images, most particles covering orthodontic wires presented spherical-like shape and nanometric size, inferior to 20nm. Is an expected result, sice hydrothermal synthesis, the method employed for AgNPs production in this work, allows controlling particles morphology and size during the synthesis [14]. Justus and coauthors [24] obtained silver nanoparticles from natural sources and observed its size around 30nm, results close to those found in this work.

More dispersed and homogeneous coating of AgNPs on the surface of Abzil® wires containing the lowest silver concentration can be explained by the greater surface smoothness and polishing existing in these wires brand. Also, it is possible that the use of higher concentrations of $AgNO_3$ (10^{-1} e 10^{-2}M) during hydrothermal synthesis causes metal saturation and agglutination on the surface of the wires.

As seen in the diffractograms, the presence of diffraction peaks at the same angles in all analyzed groups can be justified by the presence of characteristic phases of the studied material and not by the presence of AgNPs on the surface of the strands [23], so it can be inferred that the hydrothermal process did not significantly alter the profiles of the yarn segment diffractograms.

Just like in XDR, DSC curves showed that the presence of silver nanoparticles did not alter the properties of the wires. In one study, AgNPs increased thermal stability of the composites produced [25], but in a second work the incorporation of these nanoparticles in dental acrylic resins did not affect its thermal behavior [26], as well as this study. The results ensure the use of hydrothermal synthesis for the production of antimicrobial orthodontic wires.

Abzil® wires were effective against *S. aureus* and *S. mutans*, while Orthometric® wires were not. This can be explained by Orthometric® surface roughness observed in the MEV images. Therefore, in the wires of this brand, most nanoparticles were retained inside the cavities and the threads showed superficial imperfections. That compromises the effectiveness of the antibacterial action of the samples, due to the direct contact between nanoparticles surface and the microbial membrane [8,27].

The antibacterial action of metallic nanoparticles and metal oxides, such as titanium oxide, tungsten nanoparticles, cooper oxide and silver nanoparticles, for example, has been proven in some studies with orthodontic materials for the purpose of preventing or combating caries, and silver has been showing encouraging results [28-32]. Mhaske and coauthors [1] agree that orthodontic wire coating with silver may prevent the development of dental plaque and cavities during orthodontic treatment. In their in vitro study, stainless steel and nickel-titanium (NiTi) wires were coated with silver by the thermal evaporation method, and the number of bacteria responsible for cavity progression (*L. acidophilus*) adhered to the coated wires was lower, in a statistically significant way. Incorporated dental cements with AgNPs through concentrated solutions also demonstrated in vitro bactericidal activity against *S. mutans*, accentuated by the presence of nanoparticles [33].

Silver nanoparticles were incorporated into orthodontic adhesives, and have shown a significant decrease in the bacterial growth rate around the brackets, in vitro, without altering the properties of the materials [34]. Another similar study using adhesives coated with AgNPs showed inhibition of the growth of *S. mutans* on its surface. However, changes occurred in its physicochemical properties [30]. In a more recent study, brackets were coated with AgNPs by a method using $AgNO_3$ and gallic acid, and all samples tested inhibited the adhesion of *S. mutans*, with increased activity reported for the smaller particles [35]. More tests are needed, however, AgNPs showed excellent effect against adhesion of one of the major caries-causing bacteria, *S. mutans*, and biofilm formation [4,36] therefore its use for orthodontic wires coating is promising.

CONCLUSION

As hydrothermal synthesis did not alter physical-chemical properties of the orthodontic wires and allowed the dispersion of the nanoparticles on the surface of the wires, we can consider it an efficient method to obtain and incorporate AgNPs. In conclusion, the wires obtained by this process were able to prevent bacterial adhesion and biofilm formation by *S. aureus* and *S. mutans*, being efficient for this purpose. Further tests are still required, however, as orthodontic antimicrobial wires present great potential in improving oral health during orthodontic treatment.

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Conflicts of Interest: The authors declare no conflict of interest.

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