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EFFECTIVENESS OF METHODS FOR CLEANING ARCH WIRE: AN IN VITRO STUDY

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Abstract

The aim of this study was to evaluate various methods of removing bacterial and fungus biofilm, to simulate orthodontic arch wires cleaning before reinsertion in the patients appliance. Rectangular Nickel Titanium (NiTi), Stainless Steel (SS) and Titanium Molybdenum (TMA) wires were divided into five groups, then contaminated with strains of Streptococcus mutans and Candida albicas. Four segments of each group served as control and were not contaminated. Six cleanings methods were used to remove the biofilm: cotton roll and a chemical agent (chlorhexidine, sodium hypochlorite, 70% alcohol), cotton roll and water, steel woll and immersion on enzymatic detergent. There was a control group not decontaminated Then wires were placed in broth separately, and after an incubation period the optical density (OD) was measured, observing whether there was microbial growth. A wire segment of each subgroup of SS 3M® was taken to the Scanning Electron Microscope (SEM) for visualization of the treatment response. The results were submitted to one-way ANOVA test and Tukey post-test. With the exception of 70% alcohol, the disinfection means behaved similarly regardless the type of wire. Two percent Chlorhexidine and 1% Sodium Hypochlorite totally removed the microorganisms while other agents left a high microbial concentration. Chemical cleaning is necessary to remove biofilm in orthodontic wires; 1% Sodium Hypochlorite and 2% Chlorhexidine are good disinfectants for this purpose.

Keywords: Bacteria. Hygiene. Orthodontics.

1. Introduction

The orthodontic wire is one of the main tools used in corrective orthodontic mechanics (Gaikwad et al. 2020). It remains in the buccal cavity without displacement for a period of at least three weeks. The buccal cavity is rich in microorganisms (Dewhirst et al. 2010) and the introduction of any external agent, such as orthodontic fixed appliance, modifies both quantitatively and qualitatively the microbiota, and promote greater retention of plaque and pH decrease (Anhoury et al. 2002; Bastos et al. 2004; Lessa et al. 2007). Streptococcus mutans, the primary microorganism related to dental caries (Zare Javid et al. 2020), as well as Candida albicans, an opportunistic pathogen that causes candidiasis, change their behavior in the presence of orthodontic appliance (Addy et al. 1982; Hagg et al. 2004; Leung et al. 2006; Lessa et al. 2007; Gündüz Arslan et al. 2008; Hibino et al. 2009). The microbial colonization and subsequent biofilm formation occur not only in the dental surfaces but also on other surfaces, including stainless steel (Watnick and Kolter 2000;

Anhoury et al. 2002; Eliades and Bourauel 2005; Daems et al. 2009; Baboni et al. 2010). Marques states that there is a correlation between the increase of debris on the arch wire and the increase of roughness and friction (Marques et al. 2010). Therefore, when the orthodontic wire must be removed from the oral cavity between appointments and reattached after adjustments, it is necessary to clean the wire, removing the debris that is formed during the exposure time in the buccal cavity (Normando et al. 2011). In order to reduce the microbial counts, we normally performed disinfection (Rutala 1990).

Although many cleaning agents are in the market, there is no consensus of the most efficient method to make a quick arch wire disinfection in the orthodontic office. This study aimed to evaluate the effectiveness of different materials for cleaning orthodontic wires.

2. Material and Methods

This article is part of the dissertation of the author Ana Carolina Portes Canongia (https://sucupira.capes.gov.br/sucupira/public/consultas/coleta/trabalhoConclusao/viewTrabalhoConclusao.jsf?popup=true&id_trabalho=101811).

In this study, 3cm length segments of “as received” 0.019” x 0.025” arch wires were used as listed on table 1, to form groups 1 to 5.

Table 1. Distribution of arch wires groups.

Orthodontic alloy	Manufacturer	Group	Number of segments
Stainless Steel (SS)	Morelli® (Sorocaba, SP, Brazil)	1	32
Nickel-Titanium (NiTi)	Morelli® (Sorocaba, SP, Brazil)	2	32
Titanium-Molybdenum alloy (TMA)	Morelli® (Sorocaba, SP, Brazil)	3	32
Stainless Steel (SS)	3M Unitek® (Monrovia, CA, USA)	4	40
Nickel-Titanium (NiTi)	3M Unitek® (Monrovia, CA, USA)	5	32

Wires segments were exposed to strains of S. mutans (ATCC 25175) and C. albicans (ATCC 10231), and each group had a control containing 4 wires segments (except group 4 that had 5 wires due to the need for a sample for scanning electron microscopy) that were not exposed to any microorganism.

The strains of bacteria and yeast used in the study were reactivated of their primary cultures separately in petri plates containing tryptic soy broth (TSB) for 48 hours; the plaques were changed every 24 hours. After activation, the microorganisms were placed in Gibbons and Nygaard Broth (Gibbons and Nygaard 1968) and the cultures were homogenized for 30 seconds using "Vortex" mixer. With the spectrophotometer (DU® 530 Life Science UV/ Vis Spectrophotometer, Beckman Coulter™), turbidity was adjusted to be equivalent to 2.1 x 109 CFU per milliliter of medium.

Wires were placed individually, using sterile tweezers, in a 5 mL tube containing 4.5 mL of Gibbons and Nygaard broth and 0.5 mL of the suspension of microorganisms. Each tube thus had a concentration of approximately 2 x 108 CFU / mL. The flasks were incubated at 37°C for 48 hours in order to create biofilm layer in the wires.

After biofilm formation over the specimens, they were reallocated to the removing biofilm test. Each wire type, separated before by brand and / or metal composition was also separated by the technique of removing biofilm (4 wires of each group for each biofilm removal technique).

Techniques (which are detailed in subsequent rows) are of biofilm removal by:

a. Friction of cotton roll with sodium hypochlorite 1% (RH);

b. Friction of cotton roll with 2% chlorhexidine gluconate (RC);

c. Friction of cotton roll with 70% alcohol (RA);

d. Immersion in enzymatic detergent (ID);

e. Friction of steel wool (SW) and;

f. Friction of cotton roll without chemical agents (R0).

Besides these groups, there was a control group (C0) and a group exposed to the microorganisms and not decontaminated (CM). It generates eight subgroups for each group of wire.

On each wire of groups RH, RA, RC and R0, two sterile cotton containing 10 drops of 1% solution of sodium hypochlorite (RH) or 70% alcohol (RA) or 2% chlorhexidine (RC) or water distilled (R0) were rubbed 3 times in a movement of back and forward. The wires of group ID were placed into tubes containing 5mL of 0.5% detergent enzyme and were submerged for 10 minutes (according to manufacturer's instructions). The wires that were part of the SW group had its surface rubbed 3 times by a sterilized steel wool. Then a cotton roll containing 10 drops of distilled water was rubbed three times in the same way for remnants removal in every wire of each group except the group ID and R0. In wires of group CM and ID it was pipetted 3 drops of sterile distilled water so that there was no conduction of the solution with microorganisms.

The wires were placed in 5 mL solution of Gibbons and Nygaard broth where they stayed for 24 hours, allowing recovery of the microorganisms remaining over the wires. After recovery each specimen was shaken in "Vortex" mixer and by spectrophotometry (DU® 530 Life Science UV/ Vis Spectrophotometer, Beckman Coulter ™) the turbidity of the medium was verified by generating the optical density (OD).

The cleaning methods were compared inside each group and between the different wires.

One specimen of each subgroup in Group 4 (SS 3M®) was designated for the assessment through the scanning electron microscope (SEM 6490-LV), operated at 15 kV. The wires were fixed in 2.5% glutaraldehyde for a minimum of 24 hours. After fixation, the wires were washed in 0.1 M cacodylate buffer for 5 times at intervals of 30 minutes, then dehydrated using increasing concentrations of ethanol in 35%, 50%, 70%, 90% and 100% (2 times), 10 minutes interval between each change. It was made the critical point; the samples were glued on stubs for SEM analysis with double sided carbon tape and coated with gold (BAL-TEC SCD 050 Sputter Coater). Wires were viewed by two examiners, that classified them as: (-) Wires without any or with less than 30 microorganisms in the image; (+) wires with more than 30 microorganisms, but countable; (++) wires with more than 100 microorganisms. Thus, we obtained a qualitative assessment of the effects of cleaning agents.

Statistical analysis

The results of each subsection above were statistically analyzed using the software INSTAT (www.graphpad.com). The data count of microorganisms (OD) was correlated within the group of wires to which they belonged and correlated between groups using analysis of variance one-way ANOVA and Tukey post-test.

3. Results

Table 2 shows the average value of OD for each subgroup of disinfection. In analyzing these averages, the results obtained in the range from -0.020 to 0.050 are symbolized by (-); between 0.051-0.650 by (+) and above 0.650 by (+ +). Thus, it was observed that, within these ranges, except for the 70% alcohol, each technique of decontamination has a similar effect on different wires tested. From this result, the wires of the same method of decontamination were analyzed together. Table 3 shows mean and standard deviation of results.

Table 2. Optical Density means after disinfection.

Decontamination Method/ Wire (group)	SS Morelli (1)	NiTi Morelli (2)	TMA Morelli (3)	SS 3M (4)	NiTi 3M (5)
1% Sodium Hypochlorite (RH)	0.008 (-)	0.019 (-)	0.024 (-)	-0.010 (-)	-0.011 (-)
Chlorhexidine 2% (RC)	0.005 (-)	0.024 (-)	0.013 (-)	-0.007 (-)	0.004 (-)
Alcohol 70% (RA)	0.167 (+)	0.707 (++)	0.953 (++)	0.269 (+)	0.691(++)
Detergent (ID)	0.849 (++)	0.995 (++)	0.997 (++)	0.941 (++)	0.956 (++)
Steel wool (SW)	0.942 (++)	0.997 (++)	1.027 (++)	0. 876 (++)	0.882 (++)
Distilled water (R0)	0.946 (++)	0.853 (++)	0.894 (++)	0.943 (++)	0.935 (++)
No decontamination (CM)	1.013 (++)	0.992 (++)	1.030 (++)	0.939 (++)	0.968(++)
Control (C0)	0.000 (-)	0.001 (-)	0.001 (-)	0.001 (-)	-0.001 (-)

Results from -0.020 to 0.050 (-); 0.051-0.650 (+); above 0.650 (++).

Table 3. Mean values of optical density of the subgroups of the different groups. N= 20.

Decontamination Method	RH	RC	RA	ID	SW	R0	CM	C0
Mean	0.006	0.008	0.557	0.948	0.944	0.914	0.988	-0.007
SD	0.015	0.011	0.445	0.063	0.064	0.093	0.035	0.002

All groups showed a normal distribution (Kolmogorov and Smirnov method). After statistical analysis it was seen that some decontamination methods differ statistically ($p \leq 0.5$) from others, these results are shown in table 4.

Table 4. ANOVA statistical analysis of decontamination methods optical density.

Decontamination Method	RH	RC	RA	ID	SW	R0	CM	C0
RH	____	ns	***	***	***	***	***	ns
RC	ns	____	***	***	***	***	***	ns
RA	***	***	____	***	***	***	***	***
ID	***	***	***	____	ns	ns	ns	***
SW	***	***	***	ns	____	ns	ns	***
R0	***	***	***	ns	ns	____	ns	***
CM	***	***	***	ns	ns	ns	____	***
C0	ns	ns	***	***	***	***	***	____

***P<0.01; ns = not significant.

Figure 1 shows SEM images of SS wire 3M® after each disinfection process, namely: (a) sodium hypochlorite 1%, (b) chlorhexidine 2%, (c) 70% alcohol, (d) detergent, (e) steel wool; (f) water; and the wires (g) without decontamination and (h) control. The images of the wires disinfected with 1% Sodium Hypochlorite (RH) and 2% Chlorhexidine (RC) showed few remaining cocci, probably killed. The wire disinfected by 70% alcohol showed a moderate amount of cocci and yeasts, located mainly in the wire grooves The wire disinfected by detergent (ID) features lots of microorganisms, mainly coccus in a dense colony formation. Wires in which the steel wool (SW) was used, and the ones that only water was used showed a significantly reduced amount of microorganisms, cocci, which remained mostly on imperfections in the wires. The wire without decontamination (CM) presents numerous cocci and some yeasts forming a large cluster. The control wire (C0) did not have any microorganism.

4. Discussion

The presence of microorganisms adhered to the wire, impairs the sliding (Marques et al. 2010) and contributes to gingival inflammation and the development of hyperplasia, common in orthodontic treatments (Zachrisson and Zachrisson 1972; Naranjo et al. 2006; Gong et al. 2011; Gomez et al. 2018).

The hypochlorite in all wires demonstrated high power of decontamination, as could be observed in the SEM image of the few bacteria remained, and these were probably dead because there was no microbial growth during the subsequent 24 hours of incubation. The high antimicrobial power of sodium hypochlorite has been described in several articles (Bell et al. 1989; Lima et al. 2006; Da Silva et al. 2008; Andrade et al. 2009; Pellizzaro et al. 2012; Hsieh et al. 2020; Keles et al. 2020), its operation associated with the mechanical action, was considered more effective than ordinary immersion by Pellizzaro et al. (2012) and also considered effective in this study. The corrosion of metals caused by hypochlorite must be further explored, but since this process is done quickly and cleaning is usually made a few times in the same wire, the wire surface changes occur similarly in all groups (Lima et al. 2006).

The chlorhexidine digluconate is a cationic compound which has a wide spectrum of action, acting more effectively on gram-positive bacteria such as S. mutans (Da Silva et al. 2008; Sampaio et al. 2020). Its efficiency has also been demonstrated in several studies with C. albicans (Giuliana et al. 1999; Pellizzaro et al. 2012). Bambace et al. (2003) showed that cleaning stainless steel with chlorhexidine from 0.5% was

effective in removing total of S. mutans and C. albicans. In our study, 2% chlorhexidine was effective against both microorganisms on stainless steel, nickel-titanium and beta-titanium alloy.

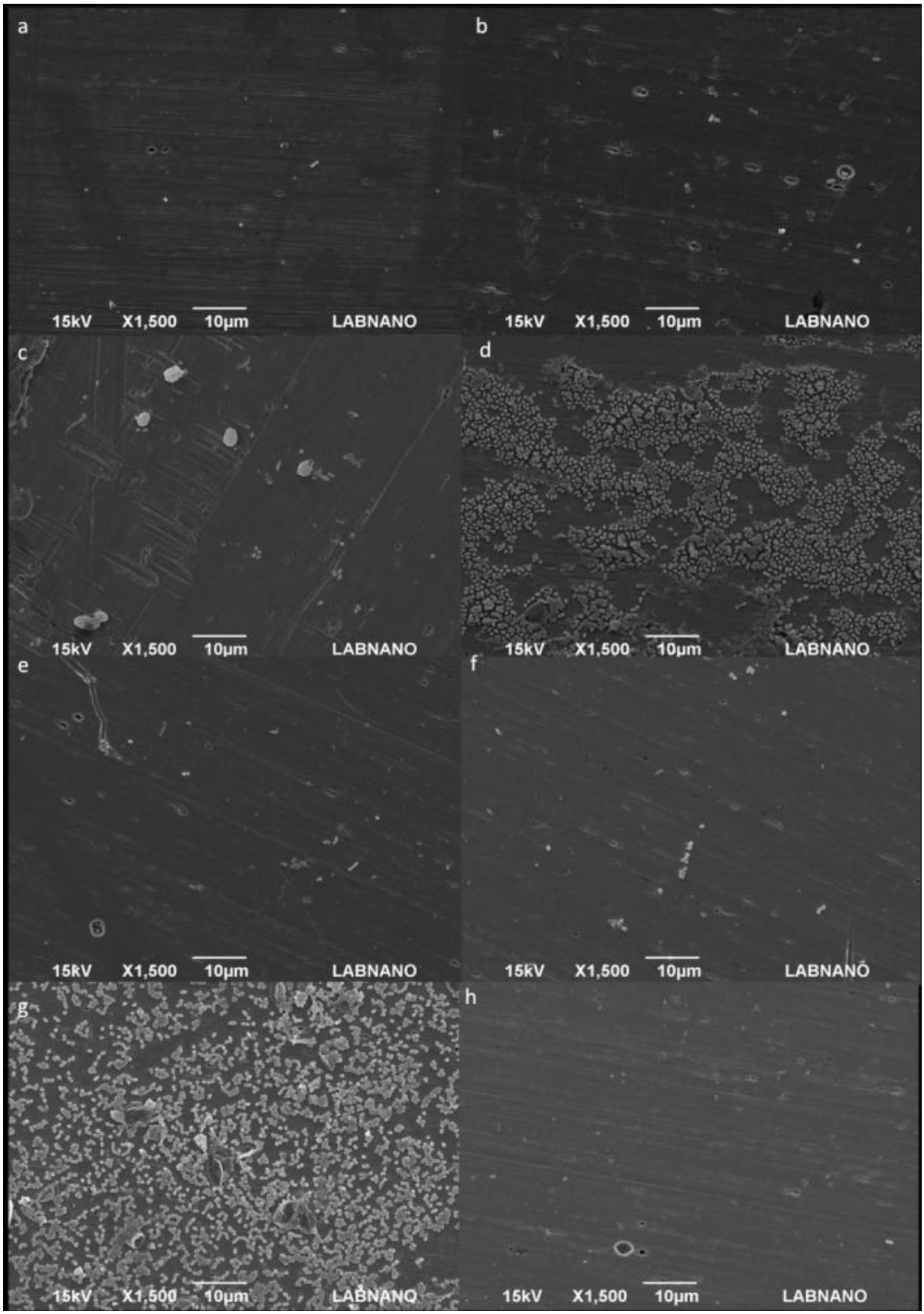

Figure 1. SEM images showing the surface of SS 3M wire after decontamination processes: a) 1% sodium hypochlorite, b) 2% chlorhexidine, c) 70% alcohol, d) Enzyme Detergent e) Steel wool f) Water . Also, the wires g) without decontamination and h) Control.

In literature, there are several reports on the use of 70% alcohol as a disinfectant as well as degerming; some encourage their use and the other contraindicate (Ayliffe et al. 1978; Myklebust 1985; Myklebust 1989; Venturelli et al. 2009; Marty Cooney et al. 2020). Just as Walder et al. (1989) article points out, alcohol fix the organic matter, leaving some microorganisms adhered still alive. In this study, alcohol results showed highly variable, 70% alcohol had a better action on SS wires, but it did not remove all microorganisms either. Our results indicated that alcohol may not be an efficient cleaner.

The enzymatic detergent showed low power disinfection, which is consistent with other studies that demonstrates this method alone is unable to remove debris or reduce microbial load to an optimal level (Lima et al. 1995). The method of immersion, as described by Pellizzaro et al. (2012) resulted in a smaller reduction of biofilm evidenced by SEM image with large number of microorganisms, Others ineffective disinfection methods, due to the mechanical aid, showed at first a microbial reduction.

The steel wool despite common being used to remove debris in stainless steel instruments, was not capable to remove the microbial layer entirely. SEM analysis showed that there was, initially, a visible reduction of the microorganisms as friction mechanically removes biofilm, but the OD viewed after 24 hours of incubation showed a high number of microorganisms. As stated previously, mechanical cleaning is itself a form to reduce microorganisms and improve friction and surface roughness (Normando, et al. 2011), but not to exterminate them. Therefore, SEM images have also showed that the group cleaned with cotton roll containing water showed a decreased amount of microorganisms, but the remaining ones grown back shortly, and it was proved that only mechanical cleaning is not effective on the long term.

The strength of this study is to analyze the best method of removing biofilm from bacteria and fungi, simulating the cleaning of the orthodontic arch wires before reinsertion into the patient's appliance. This is a frequent question among orthodontists. The negative point is due to the fact that it simulated the microorganisms in the laboratory. Ideally, future studies should make this analysis clinically.

More studies are necessary in order to determine the best way to keep orthodontic wires clean of microorganisms for a longer period of time or at least in between orthodontist appointment to reduce microbial load and improve the surface properties of the wire. A material that correctly sanitizes the orthodontic wire, however, that does not alter its properties, is a longing for orthodontics.

5. Conclusions

According to the research made all wires showed biofilm formation after infection with C. albicans and S. mutans and chemical cleaning are necessary to remove the biofilm. The most effective mechanisms for that are 1% sodium hypochlorite and 2% chlorhexidine used with cotton roll.

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