

Full Length Research Paper

Microbial contamination of orthodontic appliances made of acrylic resin

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Patients and professionals involved in dentistry are constantly exposed to potentially pathogenic microorganisms, which may be present in agents such as instruments and equipment, among others. The production of orthodontic appliances, as well as other dentistry work, is carried out in a dental laboratory where there is potential for cross-infection. Nevertheless, studies which evaluate the presence of bacterial contamination of orthodontic appliances after routine procedures in the dental laboratory are not enough. Also, there is no established clinical protocol for infection control of orthodontic appliances before they are installed in the patient. This study aimed at evaluating bacterial contamination of orthodontic appliances and the effectiveness of disinfection with 2% chlorhexidine and 0.12% chlorhexidine. Two microbiological collections were done from 60 orthodontic appliances made of chemically active acrylic resin. The first collection was made before disinfection and the second was done after, in order to evaluate bacterial growth. After analysis, it was found that 85% of sampled devices introduced were contaminated and that disinfection protocol performed with 2% chlorhexidine was effective. Furthermore, the adopted disinfection protocol should have the device soaked in 2% chlorhexidine for 10 min to prevent patient contamination from contaminated orthodontic appliances.

Key words: Orthodontic appliances, chlorhexidine, disinfection.

INTRODUCTION

The control of cross infection and biosecurity are issues of great importance to dental practice and in recent years have attracted greater interest of health professionals due to the spread of infectious diseases such as AIDS and Hepatitis B. For Russo et al. (2000) and Jorge (2002),

diseases of this kind have led to a general awareness of the risks of contamination and have changed the habits of professionals in dental clinics. These clinics have a high turnover of patients, as well as the multiple disease vector vehicles (equipment, instruments, hands of the

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operator and the environment itself), creating a serious risk of infection to the dentist, assistants and to patients (Sekijima; 1987; Venturelli; 2009).

In orthodontics, along with the high turnover of patients, there is direct contact with moldings and orthodontic impression models. According to Woo et al. (1992), orthodontists see blood in molding appointments an average of 3 times a week. This shows that disinfection of moldings that are made in offices for study and orthodontic appliance production should be of great concern, since the mold will always come in contact with patient's saliva and blood. Freitas et al. (2005) believe that there may be contamination both in the office and in the laboratory to which models/impressions are sent. Molds, impressions, prostheses or other orthodontic appliances that come in contact with patient's saliva - and therefore in contact with microorganisms settlers of oral environment such as *Streptococcus mutans*, *Streptococcus sanguinis*, *Staphylococcus aureus* and *Porphyromonas gingivalis* as related by Aguiar et al (2012) and Andrade et al (2011). Blood can serve as an indirect microorganism transmission route to laboratory technicians and the instruments they use. In this sense, Silva et al. (2010) and Pavarina (1999) claim that if adequate disinfection procedures are not implemented in the laboratory, micro-organisms may be transferred back from the laboratory to the patient, via prosthesis or appliances.

Recent studies as Jagger et al.(1995), Powell et al.(1990) and Silva et al.(2010) show that procedures such as immersion of alginate molds and plaster models in 1% sodium hypochlorite for 10 min are not carried out correctly in laboratories, not taking into consideration the concentration of the disinfectant substance as well as the optimum time for immersion. There is communication gap between dentists and technicians in regards to the disinfection of appliances, which further aggravates the situation.

Therefore, in this study the presence of contaminated orthodontic appliances made of acrylic resin was examined. Once contamination was confirmed, the efficacy of chlorhexidine in two different concentrations was evaluated, using a specific clinical protocol for infection control of these devices before they are installed in the patient.

MATERIALS AND METHODS

The study was an *in vitro* experimental method thus, the approval of ethics committee was not necessary. The sample consisted of 60 impressions/orthodontic appliances which were randomly divided into two groups of 30. All orthodontic devices included in the sampling were made of chemically activated acrylic resin.

Data collection for the evaluation of contamination in orthodontic appliances was carried out in two stages: the first was to collect the appliances in orthodontic laboratories, taking them to the microbiology laboratory of a dental school; and then, the contamination analysis was carried out in that laboratory. The devices were collected directly from the participating research

laboratories (the same way they would be sent to their orthodontist), and were then transported to the microbiology laboratory. After the experiment was completed, the appliances were stored back in the same container in which they would be delivered to the orthodontist.

Once in the laboratory, the devices were manipulated using sterile tweezers for sampling and individually deposited in a sterile saline solution (physiological saline solution), with sufficient volume to cover the entire surface. After 5 min, the container with saline was placed in a vibrator to mix the solution and two aliquots of 0.1 ml, then pipetted into each sample were seeded (surface seeding) into two Petri dishes (0.1 ml in each), containing blood-agar culture. The plates were incubated for 48 h and were subsequently analyzed for the presence of bacterial colony forming units (CFU). The counts for each plate were performed separately and then the mean was calculated between the plates to determine the final CFU for each analysed unit.

After this initial phase when contamination had been confirmed, evaluation of the disinfection was then carried out. The samples were randomly distributed by a draw and then underwent two disinfection protocols, one for each group. Group 1 consisted of 30 devices that were individually immersed for 10 min in a container containing 0.12% chlorhexidine, enough to cover the entire surface. Group 2 consisted of 30 devices that were individually immersed for 10 min in a container containing 2% chlorhexidine, enough to cover the entire surface.

After immersion, the appliances were washed with distilled water to remove excess chlorhexidine and individually immersed in a 3% Tween 80 solution and 0.3% L-alpha-soybean lectin based in saline, a specific solution to neutralize the action of chlorhexidine. After a lapse of 5 min, the solution containing the device was placed in a vibrator apparatus for 10 s, then two aliquots of 0.1 ml were removed from each sample and seeded into two Petri dishes (0.1 ml in each) containing medium culture Agar blood. After 48 h required for bacterial growth, the colony forming units (CFU) were individually counted on each plate and then the arithmetic mean between the plates was calculated to determine the final number of CFU for each device examined. The efficacy of the tested antimicrobial solutions was analyzed in this way.

Data were quantitatively analyzed by comparing the samples on each plate, comparing the initial collection with the experimental group. The colonies were manually counted with the aid of a magnifying glass. Thereby the number of colonies of the sample was converted into score, based on the following parameters: Score 0 for devices with no colonies/biofilm; Score 1 for devices presenting from 1 to 100 CFU; score 2 for 101 to 1000 CFU and score 3 more than 1000 CFU. These last three scores were used to analyse the efficacy of 0.12 and 2% chlorhexidine for appliance disinfection.

Descriptive statistical analysis was performed and verification of statistical difference conducted with the non-parametric Mann-Whitney test for variables that did not maintain dependence, the Wilcoxon test for paired variables and Fisher Exact test to assess the association, all with significance levels of 95%.

RESULTS

After complete data collection, it was observed that 10 different types of orthodontic appliances were part of sampling: Haas Expander, Haas Expander with digital springs, OAR with platinum grid, OAR with palatal crib and around expander, with OAR around expander, OAR with around expander and digital springs, Balters Bionator, Hawley plate, Hawley plate with anterior acrylic stopper/plug and Hawley card with digital spring. Sixty appliances were included in the first analysis (in which

Table 1. CFU assessment in cases disinfected with 0.12% chlorhexidine.

Parameter	n	Average (\pm SD)	Median	Q25-75	p
Initial CFU Score	30	1.97 (\pm 0.964)	2.00	1.00-3.00	0.001
Final CFU Score	30	2.67 (\pm 0.547)	3.00	2.00-3.00	

CFU, Colony-forming unit; SD, standard deviation; Q, quartile. Source: Original compilation.

Table 2. CFU assessment in cases disinfected with 2% chlorhexidine.

Parameter	n	Average (\pm SD)	Median	Q25-75	p
Initial CFU Score	30	1.03 (\pm 0.850)	1.00	0.75-1.00	<0.001
Final CFU Score	30	0.13 (\pm 0.571)	0.00	0.00-0.00	

CFU, Colony-forming unit; SD, standard deviation; Q, quartile. Source: Original compilation.

Table 3. Chlorhexidine concentration associated to disinfection.

Parameter	Disinfection (CFU score reduction)		No disinfection		Total		p	pfRP (IC:95%)
	n	%	n	%	n	%		
Use of 0.12% chlorhexidine	2	7.2	26	92.8	28	100	<0.001	24.7 (3.6-170.1)
Use of 2%chlorhexidine	22	95.6	1	4.4	23	100		

CFU, Colony-forming unit; SD, standard deviation; Q, quartile. Source: Original compilation.

the devices were infected before installation in the patient). For the second assessment only 15% were free of contamination from the start and has not been included at this stage. On the other hand 51 of the 60 appliances were included (equivalent to 85% of the sample). Of this total, 28 were in the first (disinfection with 0.12% chlorhexidine), and 23 in the second group (disinfection with 2% chlorhexidine).

The efficacy of chlorhexidine concentration of 0.12% and 2% in the reduced number of CFU is described in Tables 1 and 2. The difference of number of CFU increased when chlorhexidine 0.12% was used and decreased when 2% chlorhexidine was tested was statistically significant (Table 3).

DISCUSSION

The high initial contamination of the sample (85%), probably due to cross-infection, is well known in dental offices. While molding, the alginate mold usually come in direct contact with secretions from the patient, saliva and even blood, and if no decontamination protocols are carried out after the molding, the plaster is then poured over this contaminated alginate. As a result, these secretions that come from the patient come into direct contact with the plaster model, which then is sent to the

laboratory for preparation of orthodontic appliances. According to Ferreira (1995) and Silva et al. (2010), prosthetic/orthodontic laboratories believe they are not exposed to biological material and therefore disregard disinfection protocol because they do not have direct contact with the patient. In a study conducted in São Paulo with 60 laboratories, Cotrim et al. (2001) found that 63% of prosthetic laboratories believed in the possibility of contamination between office and laboratory, however, mold disinfection were not performed by 78% of those interviewed.

The appliances produced in the orthodontic laboratories may have more microorganisms at the end of the manufacturing process. This occurs because infecting microorganisms are found in the instruments used to produce appliances in the laboratories, and also inside the water used to accelerate the resin polymerization process (Barker, 2014).

According to the Ministry of Health Reports (MHR), Brazil (2000), disinfection in molding materials should be performed. For alginate, iodophors or 1% sodium hypochlorite immersion or spraying for up to 10 minutes should be used; for elastomers, immersion in 2% glutaraldehyde for 10 min and for zinc oxide / eugenol impression paste, immersion in sodium hypochlorite for 1:10 or 2% glutaraldehyde for 10 min. MHR also recommended disinfection of casts by spraying or

immersion in 1% sodium hypochlorite for 10 min. The importance of incorporating these behaviors in everyday clinical procedure is not limited to the prevention of material handling contaminated by the dentist, but also the possibility of cross infection between the work site and the technician. This may cause the dissemination of these microorganisms by dental prosthetic technician to prosthetic or orthodontic appliances of other patients, and also cross-infection back to the patient (Wang et al., 2007).

Been aware of these risks, the process of disinfection and sterilization of materials used in the manufacture of prostheses and orthodontic appliances must be reinforced. Some protocol pre- and/or post manufacture of disinfecting work should also be established as a preventive measure in controlling cross infections (Wang et al., 2007). According to the results of our research, it was found that the contamination of appliances exists and the possibility of cross infection is a reality. Thus, the disinfection of these devices is necessary before they are installed in patients as a way to curb and not further extend the possibility of a cross-infection cycle.

However, sterilization of orthodontic appliances made of acrylic by physical methods is not feasible because the boiling point of the monomer which composes the acrylic resin is 103.3°C and the heat distortion temperature is relatively low (95°C). Thus, according to Asad et al. (1993), Rodrigues et al. (1994) and Oliveira et al. (2007), the use of chemical disinfection is necessary for proper control of cross infection.

According to Jorge (2002), chlorhexidine (one of the chemical substances used for disinfection) acts on bacteria by breaking the integrity of their cytoplasmic membranes; resulting in loss of vital cellular constituents such as nucleic acid and potassium. Siqueira et al. (1998) have shown that in this way, although chlorhexidine kills vegetative forms of bacteria, it does not demonstrate effectiveness against spores except at elevated temperatures.

Despite the chemical effects of chlorhexidine, it is important to note that the concentration of the antimicrobial agent is a key factor for its action on microorganisms (Feist and Michele 1989). In this study, it was observed that the initial number of CFUs of 0.12 and 2% chlorhexidine were different, and the degree of initial contamination level of the first group (0.12% chlorhexidine) was much higher than the initial degree of contamination of the second group (2% chlorhexidine). Therefore, in this work we were unable to perform the actual comparison using different concentrations of chlorhexidine without assessing the effectiveness of a group in relation to another. However, in practice, it has been found that 0.12% chlorhexidine was not effective. From a total of 28 cases, there was a reduction of the initial CFU score in only 2 cases, the score was maintained in 13 (same level of initial contamination and final) and the score increased in 13 (final contamination level higher than the level of initial contamination). This

does not corroborate with the study done by Peixoto (2007), which evaluated the efficacy of 0.12% chlorhexidine spray reducing contamination by *Streptococcus mutans* group in the acrylic surface of removable orthodontic appliances. It noted through two protocols that both of them (use of chlorhexidine once or twice a week) showed efficacy in reducing contamination of acrylic surface by *mutans Streptococcus in vivo*.

Perdiza (2009) also found that the use of 0.12% chlorhexidine digluconate spray solution used twice a week significantly reduces the level of contamination of periodontopathogenic and cariogenic microorganisms. We attribute the increase in the final result of 0.12% chlorhexidine group to the possible contamination of this product and its low concentration is not be able to eliminate or reduce its own micro-organisms.

In periodontics and orthodontics, 0.12% chlorhexidine is commonly used for intraoral disinfection because usually this concentration is enough to attack periodontopathogenic microorganisms (Lessa et al., 2007). The 2% chlorhexidine is used in more severe cases of periodontal diseases or bad oral hygiene habits. In the present study, the 2% chlorhexidine may have obtained better results due to the acrylic resin surface's accumulation of microorganisms; because there were many microscopic fissures and imperfections. The surface was nonscaling which also contributed to the *mutans Streptococcus* accumulation (Lessa et al., 2007).

The result of the second group (disinfection by 2% chlorhexidine) corroborates the work of Bambace et al. (2003) which established the efficacy of chlorhexidine aqueous solutions for the disinfection of dental office surfaces at different concentrations (0.5, 1, 2, 3 and 4%) compared to 70% ethanol gel and liquid. The study found that aqueous solutions of chlorhexidine from 1% concentration showed greater efficacy in disinfecting surfaces when compared to 0.5% chlorhexidine aqueous solution and 70% alcohol gel and liquid. Despite the results obtained in the present study, it is suggested that more research works with different concentration of chlorhexidine solution that can complement this study should be carried out.

Conclusion

It could be concluded from the findings of the present study that 85% of appliances made from acrylic resin presented infection post-manufacture in specialized laboratory cases. Also, disinfection with 2% chlorhexidine, the highest concentration examined in this study, had a statistically significant efficacy, disinfecting appliances in 91.3% of cases.

Conflict of Interests

The authors have not declared any conflict of interests.

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