



IN VITRO EVALUATION OF EFFECTIVENESS IN REDUCING BACTERIAL PLAQUE OF ANTIMICROBIAL SUBSTANCES IN PATIENTS TREATED WITH ORTHODONTIC APPLIANCES

Vizitiu Th.-C., Ionescu Ecaterina

Department of Orthodontics and Dento-Facial Orthopedics, Faculty of Dental Medicine, University of Medicine and Pharmacy "Carol Davila" – Bucharest

Abstract. Orthodontic devices create, in the absence of adequate prophylactic measures, favorable conditions for the development of dental plaque. The aim of this study was to evaluate the effectiveness in reducing the bacterial load of the disinfectants from commercially available mouthwashes that are currently used by orthodontic patients. An experimental model has been developed which was used to investigate the action of antimicrobials contained in three commercial mouthwashes on microorganisms (total bacteria culture and *Streptococcus mutans* culture) isolated from dental plaque and cultured in vitro on the surface of orthodontic devices and growth media. Mouthwashes used in the experiment were three commercial products containing: 1) herbal extracts (essential oils): eucalyptol - 0.09%, menthol - 0.04%, thymol - 0.06%, methyl salicylate - 0.06%; 2) chlorhexidine gluconate - 0.2%; 3) sodium fluoride - 225 ppm. The experiments used in this study proved that chlorhexidine, whose application has drastically reduced the CFU/ml number for both total bacteria and *S. mutans*, has the biggest effectiveness of the tested antimicrobials. The next place, for the total bacterial flora, was the mouthwash containing extracts of plants and the lowest antimicrobial activity was recorded for the mouthwash containing sodium fluoride. For the *Streptococcus mutans* culture, sodium fluoride ranked second in effectiveness after chlorhexidine, and the mouthwash with herbal disinfectants had the lowest activity. Decrease of bacterial load in the presence of the three mouthwashes underlines the importance of rigorous preventive measures during orthodontic treatment, reducing the risks of dental demineralization and periodontal inflammation.

Keywords: *Streptococcus mutans*, total bacteria culture, chlorhexidine, sodium fluoride, mouthwash

Introduction

Orthodontic devices create, in the absence of adequate prophylactic measures, favorable conditions for the development of dental plaque, by adding retentive spaces, and thus increase the number of bacteria involved in the pathological processes of the oral cavity. Therefore, demineralization of enamel and gingival inflammation may occur more frequently in the case of orthodontic patients with poor hygiene [1]. In order to prevent

these pathological processes it is recommended to rigorously use prophylactic products.

The aim

The aim of this study was to evaluate the effectiveness in reducing the bacterial load of the disinfectants from commercially available mouthwashes that are currently used by orthodontic patients.

Materials and methods

An experimental model has been developed which was used to investigate the action of antimicrobials contained in three commercial mouthwashes on microorganisms isolated from dental plaque and cultured in vitro on the surface of orthodontic devices and growth media.

Theodor-Cristian Vizitiu

*Dep. of Orthodontics and Dento-Facial Orthopedics
University of Medicine and Pharmacy "Carol Davila"
email: theodorvizitiu@yahoo.com*

Mouth rinses used in the experiment were three commercial products containing the following active ingredients:

1. mouthwash with herbal extracts (essential oils) eucalyptol - 0.09%, menthol - 0.04%, thymol - 0.06%, methyl salicylate - 0.06%;
2. chlorhexidine mouthwash: chlorhexidine gluconate - 0.2%;
3. mouthwash containing sodium fluoride: sodium fluoride - 225 ppm.

Experiment I

Two bacterial cultures on Columbia agar with 5% sheep blood (one total bacterial culture and one *Streptococcus mutans* culture) have been obtained from samples of dental plaque collected from young patients wearing orthodontic appliances. The bacterial colonies from these cultures were subcultivated on liquid growth media (BHI broth) to a concentration of 10^8 CFU/ml (colony forming units /ml).

Four similar in size and shape fragments of orthodontic fixed device (Fig.1), each composed of a stainless steel bracket, an elastic module and a piece of round Ni-Ti wire, have been sterilised and immersed in each subculture and left for 48 hours at 37°C incubation, to allow the bacteria to adhere to their surface.



Figure 1. Fragment of orthodontic appliance (metal bracket, elastomeric module, Ni-Ti round wire)

After the 48 hours, the fragments of orthodontic appliances have been removed from the BHI broth culture and three of them were placed for 1 minute in 200 ml of one of the tested mouthwashes and one in 200 ml of sterile saline solution, used as control. Each fragment was then introduced in 10 ml of

sterile saline solution and ultrasonic detachment of germs adhered to the surface was performed. From the suspension obtained in each tube, decimal dilutions were made up to 10^{-9} ; 0.1 ml of each dilution was plated on GS Columbia media and incubated at 37°C in 5% CO₂ atmosphere for 18 hours, in order to determine the number of colony forming units developed on each sample.

Experiment II

From total bacteria culture and *Streptococcus mutans* culture, two suspensions in sterile saline solution with optical density of 1 and 0.5 Mc Fairland units (uMFL) were made. Each suspension was plated on a Petri dish with Mueller Hinton agar with 7% blood. On the surface of the plates, sterile discs of filter paper impregnated with 1 microliter of the three disinfectants were applied. Plates were incubated at 37°C for 18 hours, then the bacterial growth inhibition zone was measured (Fig. 2).



Figure 2. The inhibiting action of antimicrobial substances on development of the bacteria plated on growth media

Results

Results obtained in experiment I, presented in Table 1, reveal that use of mouthwashes was followed by significant decrease in bacterial load both for total flora culture and *Streptococcus mutans* culture, compared to the control sample (saline solution). For total flora culture, the CFU/ml number was most inhibited (at 17×10^2) in the case of mouthwash with chlorhexidine, at 20×10^4 for herbal essential oils and least inhibited (at 44×10^6) when

Antimicrobial substance	Bacteria count (CFU/ml)	
	Total oral flora culture	S. mutans culture
Chlorhexidine	17×10^2	34×10^2
Sodium fluoride	44×10^6	26×10^4
Herbal extracts	20×10^4	13×10^6
Control sample	22×10^7	35×10^7

Table I. The effect of antimicrobial substances on biofilm formation on orthodontic devices

Antimicrobial agent	Diameter of growth inhibition zone (mm)			
	Total oral flora culture		Streptococcus mutans culture	
	1uMFL	0.5uMFL	1uMFL	0.5uMFL
Chlorhexidine	20	29	23	31
Sodium fluoride	7	9	11	18
Herbal extracts	9	11	8	14

Table I. Bacterial growth inhibition produced by the antimicrobial agents

using mouthwash with sodium fluoride. In the case of *S. mutans* culture, bacterial growth was inhibited most with chlorhexidine (34×10^2 CFU/ml), followed by sodium fluoride (26×10^4 CFU/ml). The weakest effect on the *S. mutans* bacterial growth (35×10^7 CFU/ml) occurred when the herbal disinfectants mouthwash was used. The data obtained are in agreement with those communicated by other authors [2,3,4,5] that have demonstrated the role of various substances contained in mouth rinses in reducing the total number of bacteria in dental plaque and *S. mutans*.

Table 2 contains data recorded after applying mouthwashes on total oral flora confluent bacterial culture and *Streptococcus mutans* culture (experiment II). Using the diffusimetric method, the diameter of growth inhibition zone of the total oral flora bacterial culture had maximum values for chlorhexidine (20mm, 29mm, respectively, depending on inoculum concentration), followed by antibacterial substances extracted from plants (9mm and 11mm). Lowest diameter values of bacterial growth inhibition zones were recorded for sodium fluoride (7mm for the 1uMFL inoculum and 9mm for the 0.5uMFL one). For the Streptococcus mutans culture, the growth inhibition was the strongest for chlorhexidine (23mm, 31mm respectively). A medium inhibition was obtained using the sodium fluoride mouthwash (11mm and 18mm) and the smallest diameter of the zone of growth inhibition was present for the herbal extracts mouthwash (8mm for 1uMFL and 14mm for 0.5uMFL inoculum).

Discussion

Both experiments used in this study proved that chlorhexidine, whose application has drastically reduced the CFU/ml number for both total bacteria and *S. mutans*, has the biggest effectiveness of the tested antimicrobials, as confirmed by numerous studies in the literature [1,2,3,4,5,6,7]. The next place, for the total bacterial flora, was the mouthwash containing extracts of plants and the lowest antimicrobial activity was recorded for the mouthwash containing sodium fluoride. For the *Streptococcus mutans* culture, sodium fluoride

ranked second in effectiveness after chlorhexidine, and the mouthwash with herbal disinfectants had the lowest activity.

Published data in the literature are in agreement with results obtained in these experiments. A survey conducted by Sari and Birinci [8] reported a significant decrease in the level of *S. mutans* after a week from the introduction of chlorhexidine gluconate 0.2% in oral hygiene. Kulkarni and Damla evaluated the efficacy of chlorhexidine, sodium fluoride and triclosan [9] on a group of 60 subjects and concluded that sodium fluoride is less effective than chlorhexidine in reducing *S. mutans* levels in saliva. A study at the University of Richmond, Virginia [10] aimed at evaluating the mouthwashes efficiency highlighted the beneficial role of mouthwashes containing chlorhexidine and of those with essential oils (plant extracts) on gingivitis and dental plaque formation.

Conclusions

- Antibacterial substances in the composition of the 3 mouthwashes had the effect of reducing bacterial load (number of CFU/ml) for total oral flora culture and *Streptococcus mutans* culture.
- The most reduced effect in the case of total oral bacterial flora was recorded for sodium fluoride.
- Antibacterial substances in plants had minimal activity on *Streptococcus mutans* culture.
- Chlorhexidine has proven to be the most active substance in reducing the number of colony forming units / ml, which confirms the usefulness of the recent inclusion of this substance in the composition of orthodontic products (vanish, adhesive systems etc.).
- Decrease of bacterial load in the presence of the three mouthwashes underlines the importance of rigorous preventive measures during orthodontic treatment, reducing the risks of dental demineralization and periodontal inflammation.

ACKNOWLEDGEMENT: „This paper is supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/6/1.5/S/S17”

REFERENCES

1. Derks A, Katsaros C, Frencken JE, van't Hof MA, Kuijpers-Jagtman AM. Caries-inhibiting effect of preventive measures during orthodontic treatment with fixed appliances *Caries Res.* 2004 Sep-Oct; 38(5): 413-420.
2. Chin MY, Busscher H J, Evans R, Noar J, Pratten J., Early biofilm formation and the effects of antimicrobial agents on orthodontic bonding materials in a parallel plate flow chamber, *The European Journal of Orthodontics*, 2005, 22: 1-7.
3. Cortizo MC, Lagares ME, Fernández M., Bacterial biofilms formed in vitro and in vivo on orthodontic appliances. Effect of antimicrobial agents, *Revista CENIC Ciencias Biológicas*, 2006, 37(3): 159-161.
4. Vierrou AM, Manwell MA, Zamek RL, Sachdeva RC, Tinanoff N., Control of *Streptococcus mutans* with topical fluoride in patients undergoing orthodontic treatment, *Journal of the American Dental Association*, 1986, 113(4): 644-646
5. Petersson LG, Maki Y, Twetman S, Edwardsson S. *Mutans streptococci* in saliva and interdental spaces after topical applications of an antibacterial varnish in school children., *Oral Microbiol Immunol.*, 1991 Oct;6(5): 284-287.
6. Carolina Freire de Carvalho Calabrich, Marcelo de Castellucci e Barbosa, Maria Regina Lorenzetti Simionato, Rogério Frederico Alves Ferreira., Evaluation of antimicrobial activity of orthodontic adhesive associated with chlorhexidine-thymol varnish in bracket bonding, *Dental Press J. Orthod.*, July/Aug.2010, 15(4)
7. Cleghorn B, Bowden GH. The effect of pH on the sensitivity of species of *Lactobacillus* to chlorhexidine and the antibiotics minocycline and spiramycin., *J Dent Res.*, 1989 Jul;68(7):1146-1150.
8. Sari E., Birinci I., Microbiological evaluation of 0.2% Chlorhexidine Gluconate mouth rinse in orthodontic patients, *Angle Orthod.* 2007 Sep;77(5):881- 884.
9. Kulkarni VV, Damli SG, Comparative evaluation of efficacy of sodium fluoride, chlorhexidine and triclosan mouth rinses in reducing the *mutans streptococci* count in saliva : an in vivo study., *J Indian Soc Pedod Prev Dent.* 2003 Sep;21(3):98-104.
10. Gunsolley JC, Clinical efficacy of antimicrobial mouth-rinses., *J Dent.* 2010 Jun;38 Suppl 1:S6-10.