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

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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.
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.

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 McFarland 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).

<table>
<thead>
<tr>
<th>Antimicrobial substance</th>
<th>Total oral flora culture</th>
<th><em>S. mutans</em> culture</th>
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</thead>
<tbody>
<tr>
<td>Chlorhexidine</td>
<td>$17 \times 10^2$</td>
<td>$34 \times 10^2$</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>$44 \times 10^4$</td>
<td>$26 \times 10^4$</td>
</tr>
<tr>
<td>Herbal extracts</td>
<td>$20 \times 10^4$</td>
<td>$13 \times 10^4$</td>
</tr>
<tr>
<td>Control sample</td>
<td>$22 \times 10^7$</td>
<td>$35 \times 10^7$</td>
</tr>
</tbody>
</table>

**Table 1.** The effect of antimicrobial substances on biofilm formation on orthodontic devices
using mouthwash with sodium fluoride. In the case of S. mutans culture, bacterial growth was inhibited most with chlorhexidine (34x10^2 CFU/ml), followed by sodium fluoride (26x10^4 CFU/ml). The weakest effect on the S. mutans bacterial growth (35x10^7 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.

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REFERENCES


8. Sari E., Birinci İ., Microbiological evaluation of 0.2% Chlorhexidine Gluconate mouth rinse in orthodontic patients, Angle Orthod. 2007 Sep;77(5):881-884.
