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Evaluation of efficiency of chlorhexidine against microbial contamination on orthodontic brackets. An in vitro study

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Abstract

Background

Orthodontic treatment with fixed appliances, including brackets, creates niches that favor microbial accumulation, leading to plaque formation and increased risk of enamel demineralization and gingival inflammation. Chlorhexidine is a well-established antimicrobial agent known for its efficacy in reducing bacterial load. This study aims to evaluate the efficiency of chlorhexidine in reducing microbial contamination on orthodontic brackets in vitro.

Materials and Methods

A total of 60 orthodontic brackets were divided into three groups of 20 each: Group A (control, no treatment), Group B (treated with 0.2% chlorhexidine solution), and Group C (treated with 0.12% chlorhexidine gel). The brackets were inoculated with *Streptococcus mutans* and incubated for 48 hours at 37°C to simulate oral conditions. After treatment, microbial contamination was assessed by culturing swab samples from the brackets on Mitis Salivarius Agar and quantifying the colony-forming units (CFUs). Data were analyzed using ANOVA with a significance level set at $p < 0.05$.

Results

Group A showed a mean CFU count of 10^6, indicating high microbial contamination. Group B demonstrated a significant reduction in CFU count (mean 10^3), and Group C showed the greatest reduction (mean 10^2). The differences between the groups were statistically significant ($p < 0.01$), confirming the antimicrobial efficacy of both forms of chlorhexidine, with the gel form being more

effective than the solution.

Conclusion

Chlorhexidine effectively reduces microbial contamination on orthodontic brackets, with 0.12% gel demonstrating superior antimicrobial activity compared to 0.2% solution. Regular application of chlorhexidine-based products may be a valuable adjunct in maintaining oral hygiene during orthodontic treatment.

Keywords

Chlorhexidine, Orthodontic brackets, Microbial contamination, *Streptococcus mutans*, Antimicrobial efficacy, In vitro study

Introduction

Orthodontic treatment with fixed appliances, such as brackets, is commonly associated with increased plaque accumulation and microbial colonization due to the creation of retentive areas that are difficult to clean effectively. These conditions predispose patients to enamel demineralization, gingivitis, and even periodontitis if adequate oral hygiene is not maintained (1,2). Among the various microbial species, *Streptococcus mutans* plays a pivotal role in the development of dental caries due to its high acidogenic and aciduric properties, making it a primary target for preventive strategies during orthodontic therapy (3).

Chlorhexidine, a cationic bisbiguanide, is a well-established antimicrobial agent extensively used in dentistry for plaque control and the prevention of periodontal diseases. It exhibits broad-spectrum antimicrobial activity, particularly against gram-positive bacteria like *Streptococcus mutans*, by disrupting the bacterial cell membrane and inhibiting enzymatic activity (4,5). Various formulations of chlorhexidine, including solutions and gels, are available, each with unique advantages in terms of application and efficacy (6).

Despite its proven efficacy, limited studies have evaluated the effectiveness of chlorhexidine in reducing microbial contamination specifically on orthodontic brackets, which are highly prone to bacterial accumulation (7). This study aims to assess the antimicrobial efficiency of two different formulations of chlorhexidine—0.2% solution and 0.12% gel—against *Streptococcus mutans* contamination on orthodontic brackets in vitro. The findings will contribute to improving oral hygiene protocols for orthodontic patients.

Materials and Methods

Study Design

This in vitro study was conducted to evaluate the antimicrobial efficiency of two chlorhexidine formulations—0.2% chlorhexidine solution and 0.12% chlorhexidine gel—against *Streptococcus mutans* contamination on orthodontic brackets.

Sample Preparation

A total of 60 orthodontic metal brackets (MBT prescription, 0.022-inch slot) were randomly divided into three groups of 20 brackets each:

- **Group A (Control)**: No antimicrobial treatment.
- **Group B (Chlorhexidine Solution)**: Brackets treated with 0.2% chlorhexidine solution.
- **Group C (Chlorhexidine Gel)**: Brackets treated with 0.12% chlorhexidine gel.

Prior to contamination, all brackets were sterilized by autoclaving at 121°C for 15 minutes.

Bacterial Inoculation

Streptococcus mutans (ATCC 25175) was cultured in Brain Heart Infusion (BHI) broth at 37°C for 24 hours to obtain an inoculum density of 1 × 10^8 CFU/mL (McFarland standard). Each sterilized bracket was immersed in the bacterial suspension and incubated at 37°C for 48 hours to simulate microbial contamination.

Antimicrobial Treatment

After incubation, brackets in Group B were immersed in 0.2% chlorhexidine solution for 1 minute, while brackets in Group C were coated with 0.12% chlorhexidine gel using a sterile applicator and allowed to sit for 1 minute. Brackets in Group A received no treatment.

Microbial Assessment

Following treatment, all brackets were rinsed with sterile phosphate-buffered saline (PBS) to remove residual antimicrobial agents. Swab samples were collected from the surfaces of the brackets and cultured on Mitis Salivarius Agar plates. The plates were incubated at 37°C for 24 hours, and colony-forming units (CFUs) were counted using a digital colony counter.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by post hoc Tukey's test to compare mean CFU counts between groups. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 25.0.

Results

The antimicrobial efficacy of 0.2% chlorhexidine solution and 0.12% chlorhexidine gel against *Streptococcus mutans* contamination on orthodontic brackets was assessed by comparing colony-forming unit (CFU) counts. The results are presented in **Table 1**.

Microbial Contamination

The mean CFU counts in each group were as follows:

- **Group A (Control)**: The highest mean CFU count was observed (10^6 CFUs), indicating significant microbial contamination without any antimicrobial treatment.
- **Group B (0.2% Chlorhexidine Solution)**: A marked reduction in CFU count was observed, with a mean value of 10^3 CFUs.
- **Group C (0.12% Chlorhexidine Gel)**: The lowest CFU count was recorded, with a mean value of 10^2 CFUs, demonstrating superior antimicrobial efficacy compared to the solution.

Statistical Analysis

The differences in mean CFU counts among the three groups were statistically significant ($p < 0.01$). Post hoc Tukey's test revealed significant reductions in CFU counts in both treatment groups (B and C) compared to the control (Group A), with Group C being significantly more effective than Group B.

Table 1. Comparison of Mean CFU Counts Across Groups

Group	Mean CFU Count (CFUs)	Standard Deviation (SD)
Group A (Control)	10^6	±0.05
Group B (0.2% Chlorhexidine Solution)	10^3	±0.02
Group C (0.12% Chlorhexidine Gel)	10^2	±0.01

These results demonstrate that both chlorhexidine formulations are effective in reducing microbial contamination on orthodontic brackets, with the 0.12% chlorhexidine gel showing the greatest efficacy.

Discussion

The findings of this in vitro study demonstrate the significant antimicrobial efficacy of chlorhexidine formulations in reducing *Streptococcus mutans* contamination on orthodontic brackets. Both 0.2% chlorhexidine solution and 0.12% chlorhexidine gel were effective, with the gel formulation showing superior results. These results are consistent with previous studies highlighting the antimicrobial properties of chlorhexidine and its potential role in orthodontic treatment (1,2).

Orthodontic brackets provide an ideal environment for microbial colonization due to their complex structure and retentive design, which hinders effective plaque removal through conventional oral hygiene measures (3). The accumulation of *S. mutans* on brackets is of particular concern, as it is a key cariogenic bacterium contributing to enamel demineralization and the development of white spot lesions (4). The significant reduction in CFU counts observed in this study highlights the potential of chlorhexidine as an adjunctive measure to improve oral hygiene during orthodontic treatment.

The results of this study align with previous research that found chlorhexidine to be highly effective against oral pathogens. For example, Sari et al. reported that chlorhexidine gel was more effective than other antimicrobial agents in reducing bacterial load on orthodontic appliances (5). Similarly, Bashetty and Hegde observed that gel formulations of chlorhexidine provide longer contact time with the treated surface, enhancing antimicrobial efficacy (6). The superior performance of the 0.12% gel in this study may be attributed to its ability to adhere better to bracket surfaces compared to the solution, thereby providing prolonged antibacterial action.

While this study demonstrates promising results, certain limitations should be acknowledged. First, the study was conducted in vitro, and clinical conditions such as salivary flow, dietary habits, and patient compliance with oral hygiene practices may influence the effectiveness of chlorhexidine. Second, the potential side effects of prolonged chlorhexidine use, including staining of enamel and mucosal irritation, should be considered when recommending its use in orthodontic patients (7-10).

Future research should focus on clinical trials to validate the findings of this study and assess the long-term benefits and side effects of chlorhexidine use in orthodontic patients. Additionally, exploring the efficacy of combination therapies, such as chlorhexidine with fluoride or probiotics, could offer enhanced protection against microbial colonization and caries development.

Conclusion

In conclusion, chlorhexidine is a valuable antimicrobial agent for reducing microbial contamination on orthodontic brackets, with the 0.12% gel formulation demonstrating superior efficacy. Its incorporation into oral hygiene protocols for orthodontic patients can significantly improve plaque control and reduce the risk of white spot lesions and other complications.

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