

Efficacy of Papacarie[®] in reduction of residual bacteria in deciduous teeth: a randomized, controlled clinical trial

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OBJECTIVES: The aim of the present study was to analyze the efficacy of Papacarie[®] gel compared with the traditional method (low-speed bur) in reducing the counts of total bacteria, *Lactobacillus*, total *Streptococcus* and *Streptococcus mutans* group.

METHODS: A randomized, controlled clinical trial with a split-mouth design was performed. The sample comprised 40 deciduous teeth in 20 children (10 males and 10 females) aged four to seven years. The teeth were randomly allocated to two groups: G1, or chemomechanical caries removal with Papacarie Duo[®], and G2, or the removal of carious dentin tissue with a low-speed bur. Infected dentin was collected prior to the procedure, and the remaining dentin was collected immediately following the removal of the carious tissue. Initial and final counts of bacterial colonies were performed to determine whether there was a reduction in the number of colony-forming units (CFUs) of each microorganism studied. ClinicalTrials.gov: NCT01811420.

RESULTS: Reductions were found in the numbers of total bacteria, total *Streptococcus* and *Streptococcus mutans* group following either of the caries removal methods ($p < 0.05$). A reduction was also noted in the number of *Lactobacillus* CFUs; however, this difference did not achieve statistical significance ($p > 0.05$).

CONCLUSION: Papacarie[®] is an excellent option for the minimally invasive removal of carious tissue, achieving significant reductions in total bacteria, total *Streptococcus* and *S. mutans* with the same effectiveness as the traditional caries removal method.

KEYWORDS: Dental Caries; Papain; *Streptococcus mutans*; Bacteria.

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INTRODUCTION

Minimally invasive treatment has been increasingly employed in the management of dental caries, especially in young children (1-5). Within the scope of this philosophy, the chemomechanical removal of carious tissue consists of the application of a natural or synthetic agent to dissolve the contaminated tissue and facilitate its removal with the aid of atraumatic mechanical force (6). Papacarie[®] is a gel containing papain and chloramine that is used in combination with manual tools for the minimally invasive removal

of carious tissue. This method eliminates the need for local anesthesia and the use of a bur, thereby reducing the destruction caused to sound dental tissue (2,7,8).

Several studies have investigated the efficacy of this gel and have reported satisfactory results regarding the time required for the procedure (9,10), clinical follow-up (7), complaints of pain (9,11), patient acceptance (9) and cost (9). Researchers have also tested the cytotoxicity of the substance at different concentrations in fibroblast cultures, demonstrating its safety for use in pediatric patients (12).

The papain-based gel has bactericidal and bacteriostatic properties (13), which may affect the number of microorganisms found in the dentin following the removal of carious tissue (14). No randomized clinical trials have compared this gel with rotary instruments for the removal of carious tissue. Thus, the aim of the present study was to analyze the efficacy of Papacarie[®] gel compared with the traditional method (TM) (low-speed bur) in reducing the counts of total bacteria, *Lactobacillus*, total *Streptococcus* and *Streptococcus mutans* group.

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No potential conflict of interest was reported.

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MATERIALS AND METHODS

Ethical aspects and subject selection

This study received approval from the local human research ethics committee (University Nove de Julho, process no. 219047). Parents or legal guardians received detailed information on the study and signed a statement of informed consent, permitting the participation of their children. Children seeking treatment at the dental clinic of the University Nove de Julho (Brazil) were recruited, and each subject was submitted to initial clinical and radiographic examinations. The eligibility criteria were as follows: an absence of systemic illness; adequate behavior; and active, acute caries in the dentin of at least two deciduous molars, not surpassing 2/3 of the dentin and involving only the occlusal surface, with a direct view and access and no clinical or radiographic signs or symptoms of pulp involvement. Following the application of these criteria, the sample included 40 deciduous teeth in 20 children (2 teeth per child) aged four to seven years.

This study was registered at Clinicaltrials.gov: NCT 01811420.

Study design

A randomized, controlled clinical trial with a split-mouth design was performed to compare the efficacy of Papacarie® gel (Fórmula & Ação, São Paulo, SP, Brazil) and the traditional caries removal method (low-speed bur). Randomization was performed using lots to determine which teeth would undergo which method:

- Group 1 (G1; n = 20) – chemomechanical removal with Papacarie®
- Group 2 (G2; n = 20) – traditional caries removal with a low-speed bur (control group)

The interventions were performed by a single operator who had undergone a calibration exercise (Kappa statistic: 0.9). The patients were submitted to the procedures without the prior administration of local anesthesia but were told that anesthesia was available, if needed. Both interventions followed the same initial protocol: initial periapical and interproximal radiography and prophylaxis with a Robinson brush and fluoride toothpaste, followed by relative isolation (lip bumper, cotton roll and saliva aspirator).

Dental plaque collection

A sample of infected dentin was collected from each tooth prior to the removal of the carious tissue. Sample collection was standardized with the use of a n° 2 Meyhoefer curette (Erwin Guth, São Paulo, SP, Brazil), and each specimen was deposited in a test tube containing 3.8 mL of pre-reduced, anaerobically sterilized VMGA III (Viability Medium Goteborg Anaerobically) gelatinous transport medium. After a 30-min incubation at 37°C to liquefy the gelatin in the medium, the dental biofilm was dispersed in a transport flask containing glass beads by placement on a tube shaker at maximum velocity for 30 min.

Following the collection of a sample of infected dentin, the caries removal methods were performed. In G1, Papacarie® was applied and allowed to set for 30 to 40 s, followed by the removal of the softened carious tissue using the blunt end of a curette. Reapplications were performed, if

necessary, until complete removal of the carious tissue. In G2, caries removal was performed with a low-speed bur. The procedures were terminated when no further softened dentin tissue was observed, as determined by an inspection of the texture of the remaining dentin and the use of an exploratory probe.

Immediately after the removal of the carious tissue, a sample of the remaining dentin was collected with the use of a n° 2 Meyhoefer curette, following the same procedures as described above. Restorations were performed with glass ionomer cement (Ketac Molar Easymix, 3 M ESPE™ [Dental Products, St. Paul, MN, USA]). Follow-up and radiographic control sessions were then scheduled.

Microbiological procedures

The samples were centrifuged for 5 s and decimally diluted, and 0.1 mL was plated onto the following bacteriological media: blood agar for total anaerobic and facultative anaerobic colony counts, mitis salivarius-bacitracin (MSB) agar (Difco Laboratories, Teddington, Middlesex, UK) with sucrose (15) for the isolation of *Streptococcus mutans* and Rogosa SL agar (Merck, Darmstadt, Germany) (16) for the isolation of lactobacilli. These media were incubated anaerobically (GasPak Anaerobic System, Becton Dickinson, Franklin Lakes, NJ, USA) at 37°C for 5 days. Each decimal dilution was plated onto two agar plates (MSB agar and Rogosa agar). *Streptococcus mutans* was counted on the MSB agar and biochemically identified using a previously published identification scheme. Gram-positive rods isolated from the Rogosa agar were tested for the absence of catalase and for resistance to vancomycin to identify lactobacilli. The Rogosa and blood agar plates were incubated under anaerobic conditions for 48 h, while MSA and MSB plates were incubated in a 5% CO₂ atmosphere for 48 h (15).

After incubation, the number of colony-forming units (CFUs) was determined on plates containing 10 to 300 colonies. Microbial counts were performed in duplicate with a digital colony counter (CP 600 Plus, Phoenix) by a single examiner who was blinded to the allocation of the specimens to the different groups. Individual bacterial counts were expressed as log₁₀ CFUs. The mean counts on the two simultaneously incubated Rogosa and MSB agar plates were calculated to express the individual counts of lactobacilli and *Streptococcus mutans* per dentin sample.

Table 1 - Distribution of deciduous teeth according to the type of treatment.

Tooth	CMCR	TM
54	3 (15.0)	2 (10.0)
55	1 (5.0)	1 (5.0)
64	1 (5.0)	1 (5.0)
65	5 (25.0)	2 (10.0)
74	3 (15.0)	4 (20.0)
75	3 (15.0)	4 (20.0)
84	3 (15.0)	1 (5.0)
85	1 (5.0)	5 (25.0)
Total	20 (100.0)	20 (100.0)

CMCR – chemomechanical caries removal.

TM – traditional method.

**Table 2** - Mean number of colony-forming units in each group before and after caries removal.

	CMCR			TM		
	Before	After	p-value	Before	After	p-value
Total bacteria	11.64×10 ³	1.61×10 ³	0.027	12.22×10 ³	6.8×10 ³	0.028
Total <i>Streptococcus</i>	15.79×10 ³	3.06×10 ³	0.043	14.4×10 ³	9.08×10 ³	0.028
<i>S. mutans</i>	1.33×10 ³	0.09×10 ³	0.028	1.32×10 ³	0.20×10 ³	0.027
<i>Lactobacillus</i>	1.8×10 ³	0.22×10 ³	0.226	2.63×10 ³	0.27×10 ³	0.116

CMCR – chemomechanical caries removal.

TM – traditional method.

Statistical analysis

Bacterial counts in CFUs were transformed into log₁₀. Differences in the mean number of CFUs were determined using analysis of variance (ANOVA), followed by Tukey's test. The SPSS 17 program (IBM Corp., Chicago, IL, USA) was used for the statistical analysis, with the significance level set to 5% ($p < 0.05$).

RESULTS

The sample was composed of 20 children (10 boys and 10 girls) between four and seven years of age (mean: 5.6 years). The molars were the teeth of choice. Table 1 displays the distribution of the teeth according to the type of treatment.

Table 2 displays the results of the microbiological analysis in the two groups before and after the removal of carious dentin tissue. Statistically significant reductions were found in the numbers of total bacteria, total *Streptococcus* and *Streptococcus mutans* in both groups ($p < 0.05$). A reduction also occurred in the number of CFUs of *Lactobacillus*; however, the difference did not achieve statistical significance ($p = 0.226$ with chemomechanical caries removal (CMCR) and $p = 0.116$ with TM). The CMCR group exhibited a greater reduction in bacteria compared with the control group; however, this difference also did not achieve statistical significance ($p > 0.05$).

DISCUSSION

Both caries removal methods led to statistically significant reductions in total bacteria, total *Streptococcus* and *Streptococcus mutans* group, with no statistically significant differences between the groups. The reduction in bacteria with the use of the papain-based gel may be related to the bactericidal and bacteriostatic action of the gel, which results in the inhibition of gram-negative and gram-positive bacteria (13).

The reduction in microorganisms in both groups indicates the efficacy of the two methods. These results, with the exception of the lactobacilli data and the technique comparison, are in agreement with findings described in a previous study. El-Tekeya et al. evaluated the effect of two CMCR methods (Carisolv and Papacarie®) on residual cariogenic bacteria in the dentin of deciduous teeth compared with the effects of traditional manual excavation. The results demonstrated that CMCR (Carisolv and Papacarie®) led to significant reductions in total bacteria, *Streptococcus mutans* group and lactobacilli. The authors also found that Papacarie® was significantly more effective with respect to its reduction in residual cariogenic bacteria compared with both Carisolv and manual excavation (14).

In another study, four of 15 teeth treated with Papacarie® and one of 15 teeth treated with the TM exhibited bacteria.

Despite the greater prevalence of microorganisms in the former group, the authors reported that these microorganisms were generally found in the dentin/enamel junction and that Papacarie® is effective, considering its less destructive effects on sound dentin tissue (10). This feature may be attributed to the fact that Papacarie® does not affect the collagen fibers of sound dentin, as papain degrades only dead cells (2).

Bacterial counts have been investigated with the use of other minimally invasive techniques, such as atraumatic restorative treatment (ART), which only employs manual instruments for the removal of carious dentin. Investigators have reported a reduction in microorganisms following manual cavity preparation, demonstrating the reliability of the use of standardized ART (17,18).

Based on the findings of the present study, Papacarie® is an excellent option for the minimally invasive removal of carious tissue, achieving significant reductions in total bacteria, total *Streptococcus* and *S. mutans* with the same effectiveness as that observed in the traditional caries removal method, while offering the advantage of less destructive effects on sound dental tissue.

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AUTHOR CONTRIBUTIONS

Motta LJ and Bussadori SK conceived and designed the experiments. Motta LJ, Bussadori SK, Campanelli AP, Silva AL and Navarro MF performed the experiments. Alfaya TA and Godoy CH analyzed the data. Alfaya TA and Godoy CH wrote the paper.

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