A comparative in vitro efficacy of conventional rotatory and chemomechanical caries removal: Influence on cariogenic flora, microhardness, and residual composition

Rene Garcia-Contreras1,4, Rogelio Jose Scougall-Vilchis1,2, Rosalia Contreras-Bulnes1,3, Hiroshi Sakagami5, Raul Alberto Morales-Luckie6, Hiroshi Nakajima7

1Dental and advanced Studies Research Center “Dr. Keisaburo Miyata”, 2Deparments of Orthodontics, 3Pediatric Dentistry, 4Dental Materials, School of Dentistry, 5Department of Nanomaterials, Center for Research in Sustainable Chemistry (CIQS), School of Chemistry, Autonomous University State of Mexico (UAEmex), State of Mexico, Mexico, Department of Diagnostic and Therapeutic Science, 6Division of Pharmacology, 7Biomaterials Science, School of Dentistry, Meikai University, Saitama, Japan

Abstract

Background: Chemomechanical caries removal system is part of the minimal invasive dentistry; the aim of the study was to compare the amount of bacteria after caries removal with chemomechanical system and conventional rotatory instruments and to test the Vickers microhardness and micro-RAMAN analysis of residual dentin after excavation.

Materials and Methods: Molars were induced for demineralization, confirmed with DIAGNOdent; Streptococcus mutans were inoculated into the cavities and filled. Caries removal was performed with rotatory instruments and chemomechanical system; surviving bacteria were cultured for 24 and 48 hours at 37°C. Vickers microhardness and micro-RAMAN analysis were tested after excavation. Data were analyzed with Wilcoxon, continuity correction, odds ratio, ANOVA post hoc Tukey test, and Spearman correlation.

Results: Demineralization was significantly detectable at 240 hours of incubation; conventional rotatory instruments and chemomechanical caries removal were effective in 19.4%-22.6% and 25.8%-32.3%, respectively. Vickers microhardness of chemomechanical system was higher (P < 0.0001) than conventional rotatory instruments and comparable to healthy dentin. Micro-RAMAN analysis showed that healthy dentin is correlated to chemomechanical system ($R^2 = 0.683$, $P < 0.00001$) and drilling with burs ($R^2 = 0.139$, $P < 0.00001$).

Conclusion: The chemomechanical system is effective for caries elimination, comparable to conventional rotatory instruments; the remaining Vickers microhardness and composition surface tissue are similar to healthy dentin.

Keywords: Cariogenic flora; chemomechanical caries removal; conventional rotatory caries removal; RAMAN; Vickers microhardness

INTRODUCTION

The individual health preservation of natural teeth should be the objective of every physician. Dental adhesives and restorative materials have been developed with new understanding of the caries process and remineralization, and changes in caries prevalence have catalyzed the evolution of management from G.V. Black's “extension for prevention” to “minimally invasive dentistry.” The “minimally invasive dentistry” approach to treating dental caries incorporates the dental science of detecting, diagnostic, intercepting, and treating dental caries on the microscopic level. Minimally invasive techniques include laser-ablation, sono-abrasion, and chemomechanical caries removal. The chemomechanical technique is a selective removal of caries dentin introduced in the 1970s.

Carisolv (MediTeam, Göteborg, Sweden) is a viscous substance for the chemomechanical removal of caries. The solution consists of two liquids, which are mixed before application: Liquid 1 contains mainly three amino acids.
Conventional G. V. Black class I cavities were prepared. A mounting jig was used to align each tooth labial surface. Mexico) with a label bearing the number of each sample. Molars were stored in 0.2wt/% thymol solution at 4°C. Molars were obtained from each patient who visited the clinic at the Faculty of Dentistry after signing the informed consent. The Internal University Bioethics Committee, and the patients.

RESULTS

Results have pointed that the major advantages are as follows: Decreased discomfort, improved good behavior management, minimizing the use of anesthesia, selective removal of infected tissue, exposed dentin tubules, and low risk to pulp exposure. The chemomechanical system is a promising material to treat primary teeth because it offers more comfort to the patient than conventional drilling caries removal. However, caries removal takes longer than the conventional method with burs, and the chemomechanical system leaves demineralizing dentin that can interfere with the adhesion. Nevertheless, studies focused on the resin adhesive penetration of dentin after caries removal with burs and chemomechanical was 15 and 10 μm, respectively; caries removal through the chemomechanical method induced a higher hybrid layer than conventional rotary instruments. A few studies suggested that chemomechanical caries removal with Carisolv results in an irregular surface with low rates of bacteria; the chemomechanical system yields 90.5%, without bacteria; meanwhile, drilling with burs results in higher amount of microorganisms than the chemomechanical system after culture and incubation in agar.

We hypothesized that chemomechanical caries removal with Carisolv is better than conventional rotatory burs as suggested by microbiological agar culture, dentin Vickers microhardness, RAMAN micro-analysis, and EDX analysis. The aims of this study were as follows: 1) to compare the amount of bacteria after caries removal with chemomechanical system and conventional rotary instruments; and 2) to compare Vickers microhardness and micro-RAMAN analysis of residual dentin after caries removal with both mentioned techniques.

MATERIALS AND METHODS

In vitro caries model

This experimental study design was carried out from February 2013 to July 2013. The study was approved by the Internal University Bioethics Committee, and the molars were obtained from each patient who visited the clinic at the Faculty of Dentistry after signing the informed consent. Sixty-two caries-free freshly extracted molars were stored in 0.2wt/% thymol solution at 4°C. Molars were fixed in acrylic resin (NicTone 62; MDC Dental, Guadalajara, Mexico) with a label bearing the number of each sample. A mounting jig was used to align each tooth labial surface. Conventional G. V. Black class I cavities were prepared with high-speed spherical #8 carbide bur (SS White, Gloucester, UK) by cooling. Molars were ultrasonically cleansed in distilled water (Quantrex, Kearny, NJ, USA) for 5 minutes. Samples were immersed in demineralizing solution (1.5 mM CaCl$_2$, 0.9 mM KH$_2$PO$_4$, 150 mM KCl, and 0.1 mM sodium acetate at pH 4.5) and incubated at 37°C for 360 hours. Then, the mineral content of bottom cavities were estimated at three points with fluorescence laser diagnosis (DIAGNOdent, KaVo, Biberach, Germany) before dipping to the demineralizing solution, at 180 and 360 hours. Cotton pellets were embedded with brain heart infusion (BHI; Becton Dickinson, NJ, USA) containing 10$^5$ bacteria suspension of Streptococcus mutans (ATCC 35668) and set in cavities. Molars were filled out with zinc oxide eugenol (SS White, Gloucester, UK) and incubated at 37°C by partial anerobiosis for 48 hours.

Caries removal

Molars were randomly divided into two groups (n = 31): Low-speed conventional rotatory bur and chemomechanical Carisolv system. Zinc oxide eugenol was taken out of the molars. Carious tissue was removed with spherical #6 carbide bur (SS white) under cooling by single operator. For Carisolv (single mix), solutions 1 and 2 were mixed, gel was applied and left in the cavity for 30s, and carious dentin was afterward removed with Hu-friedy curette (Chicago, IL, USA). The gel was reapplied until cavity presented the nonexistence of softened carious tissue. To gauge carious tissue removal for both conventional rotatory bur and Carisolv, an exploratory probe and caries detector (Viarden, Mexico City, Mexico) were used to check until the hard dentin was obtained.

Bacterial culture

Immediately after caries removal, 100 μl of BHI were inoculated into the cavities and curetted the surface of preparation. BHI was recollected with a pipette containing the remaining tissue and stored in 5 ml of BHI. Each sample of both groups was incubated for 24 hours at 37°C. The remaining bacteria suspended in the BHI of each sample were cultured in brain heart agar (BHI; Becton Dickinson) and incubated for 24 and 48hours. The colony-forming units were determined for total bacterial counts. Bacterial culture experiment was carried out for triplicate of each sample to obtain reproducible data.

Vickers microhardness analysis

Five randomly chosen molars of each group were sectioned horizontally on the cervical limit, mesial walls of the cavity, and transversal region (slightly over the bottom) of the cavity with low-speed diamond wheel saw (South Bay Technology, Inc, San Clemente, CA, USA) under cool temperature until a dentin block was obtained. Blocks were finished with #600, 1000, 1500, and 2000 waterproof abrasive paper (Fuji Star, Sankyo, Rikagaku, Okegawa, Japan). The
hardness of residual dentin was evaluated using Vickers microhardness testing machine (DongGuanSinowon precision instruments, Nancheng, DongGuan, China). The Vickers diamond indenter was applied to the dentin surface at 10N, and a dwelling time of 10 s was used for 10 indentations across the specimens of each group, resulting in a total of 50 indentations for all the groups. Five healthy dentin blocks were used as a positive control to contrast with the other groups under the same conditions. Data were recorded in Vickers microhardness (VHN).

### Micro-RAMAN analysis
Dentin blocks were analyzed after caries removal with conventional rotatory instruments and chemomechanical system. Healthy dentin blocks were used as positive control. To clean the specimen for both groups and positive control, all samples from each group were ultrasonically cleansed in distilled water for 5 minutes and dried out at room temperature. Then, the samples were adhered to aluminum stubs. Blocks were analyzed using the micro-RAMAN system (HR-800; JobinYvon Inc., Edison, NJ, USA) equipped with a Microscope Olympus (BX-41) and a CCD as detector; the system is equipped with He-Ne laser (emission at 632.8 nm) at a power level of 60 µW.

### Statistical analysis
All experimental procedures were carried out by one blind investigator, the statistical analysis were performed for another author (Single blind). All variables were subject to Kolmogorov-Smirnov normality test. Demineralized teeth were analyzed using Wilcoxon signed rank test. Chemomechanical and conventional caries removal were evaluated with continuity correction (Yates correction) and odds ratio. McNemar test was used to compare incubation time between groups. Vickers microhardness of the remaining dentin was analyzed using ANOVA post-hoc Tukey test, and in case of micro-RAMAN analysis, data were tested using Spearman correlation. All data were analyzed with SPSS (Version 18; SPSS, Inc., Chicago, IL, USA).

### RESULTS

#### In vitro caries model
Molars in contact with demineralized solution showed the following DIAGNO dent values: At 0 hour (55.17 ± 18.68), 120 hours (55.80 ± 28.01), and 240 hours (63.01 ± 29.92); Wilcoxon signed rank test showed significant differences in degree of demineralization. Values were tested using DIAGNODent at 240 hours of incubation with solution; when comparing 0 hour ($P < 0.05$) and 120 hours ($P < 0.001$), significant differences are shown. Among values and course time, the 0 hour and 120 hours did not show significant differences; the demineralization process was observed after 120 hours of incubation at 37°C.

#### Caries removal and Streptococcus mutans culture
Conventional rotatory and chemomechanical caries removal yield 22.6% and 32.3% of clean surface, respectively, when cultured in BHI agar for 24 hours of incubation at 37°C. There was no statistically significant difference with continuity correction test. In terms of 48 hours of incubation in BHI agar containing the remaining cavity bacteria, 19.4% and 25.8% were observed, respectively; continuity correction showed significant differences between groups ($P < 0.05$). Tables 1 and 2 show the cross-tabulation of caries removal with the two methods. McNemar test during the time course of incubation for both conventional rotatory instruments and chemomechanical caries removal did not show any difference because 24 and 48 hours of culture in BHIagar containing S. mutans did not show significant difference.

#### Vickers microhardness analysis
The mean microhardness value of blocks for conventional rotatory instruments was $60.42 \pm 4.40$ VHN; chemomechanical caries removal was $67.40 \pm 5.58$ VHN, and healthy dentin represents $66.94 \pm 4.77$ VHN. Microhardness scores followed a normal distribution. ANOVA post-hoc Tukey test showed that the microhardness of conventional rotatory instrument caries removal dentin is different ($P < 0.0001$) than that of chemomechanical caries removal dentin and that of healthy dentin; meanwhile, there are no statistically significant differences between the latter two dentins.

#### Micro-RAMAN analysis
Data obtained did not represent a normal distribution; thus, Spearman correlations were performed. Figure 1

| Table 1: Conventional rotatory instruments and chemomechanical caries removal; Streptococcus mutans (SM) BHI agar culture at 24 hours |
|---------------------------------|-----------------|-----------------|-----------------|
| **Efficacy of SM removal**      | **Conventional rotatory caries removal** |
| SM free (%)                     | SM (%)          | Total (%)       |
| Chemomechanical caries removal  | 3               | 7               | 10              |
| SM free (%)                     | 42.9            | 29.2            | 32.3            |
| SM (%)                          | 57.1            | 70.8            | 67.7            |
| Total                           | 7               | 24              | 31              |
|                                | 100             | 100             | 100             |

Continuity correction = 0.049, $P = 0.824$, Odds ratio = 1.821

| Table 2: Conventional rotatory instruments and chemomechanical caries removal; Streptococcus mutans (SM) BHI agar culture at 48 hours |
|---------------------------------|-----------------|-----------------|-----------------|
| **Efficacy of SM removal**      | **Conventional rotatory caries removal** |
| SM free (%)                     | SM (%)          | Total (%)       |
| Chemomechanical caries removal  | 4               | 4               | 10              |
| SM (%)                          | 66.7            | 29.2            | 25.8            |
| Total                           | 33.3            | 70.8            | 64.2            |
|                                | 6               | 25              | 31              |
|                                | 100             | 100             | 100             |

Continuity correction = 4.111, $P = 0.043$, Odds ratio = 10.560
shows the RAMAN micro-analysis of healthy dentin block and the two caries-removal methods.

**DISCUSSION**

This study was focused on the influence of chemomechanical caries removal by Carisolv uncolored gel compared with conventional rotatory instrument caries removal. *In vitro* studies have reported that 17.5% of patients experience pain when Carisolv is used compared with 40% when high-speed rotatory instruments excavation is used.[11]

Model caries *in vitro* here used seems to be a reproducible model to demineralized-induced and corroborate by laser diagnosis DIAGNOdent. An alternative of demineralization is pH-cycling every 3 days,[12] however, the method produced considerable demineralization.

On the other hand, *S. mutans* (10⁵) suspension cotton pellet, embedded and positioned into the demineralized cavities and filled through zinc eugenol cement incubated at 37°C with partial anerobiosis, is another option to induce biofilm bacteria, for example, tryptic soy broth[13] to induce plaque-like biofilm as well as the technique reported here. The sensibility of DIAGNOdent is 100%, and the specificity is 87%; DIAGNOdent is considering a thrust and reproducible device system comparable with histological analysis.[14,15]

The rates of caries removal with chemomechanical system have been reported to be about 88%-90.5%;[9,10,16] the data reported here, corroborated by the results of *S. mutans* culture in BHI agar after caries removal, are 32.3% and 25.5% for 24 and 48 hours of incubation, respectively. Consequently, the data are significantly less than the values previously reported. Histological studies after Carisolv excavation show the presence of bacteria into the dentinal tubules; when compared with conventional caries removal, high concentration of bacteria are shown with conventional drilling, similar results were reported in this study after agar culture of bacteria.[9] For the treatment of patients, chemomechanical caries removal is recommended because of higher removal ratio of caries, as compared with drilling with burs (71% vs. 81%).[11,17]

Previous microhardness studies after caries removal by chemomechanical and rotatory instrument demonstrated that there is no difference in hardness between the two methods when tested in permanent and primary teeth.[6,18] In this research, Vickers microhardness for conventional caries removal was 60.42VHN; chemomechanical caries removal was 67.40VHN, and health dentin represents 66.94 VHN. Because similar values were obtained between the remaining dentin of the mineralized tissues with chemomechanical caries removal and the healthy dentin, the chemomechanical system may be an effective option for caries elimination.

Studies focused on chemical composition by RAMAN and energy disperse X-ray (EDX) of remain dentin after excavation with chemomechanical and rotatory bur caries removal; no significant differences were observed.[8] Spearman correlation between chemomechanical caries removal and healthy dentin are correlated in 68% when tested with micro-RAMAN analysis, data advocate the similarity.

The adhesive interactions, hybrid layer formation, shear bond strength, topographical analysis of the remaining surfaces, and biocompatibility with the surrounding tissues should be investigated to understand the biological impact of using chemomechanical caries removal and the micro-RAMAN analysis of soft and carious dentin. Although Carisolv is used as an alternative for caries excavation, more scientific evidence is essential to consider it as an effective and safe system for minimally invasive dentistry.

**CONCLUSION**

- Chemomechanical system is effective for caries elimination comparable to conventional rotatory instruments.
- The remaining Vickers microhardness and composition surface tissue are similar to healthy dentin.

**REFERENCES**


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