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ORIGINAL RESEARCH

In vitro Remineralization of Caries-affected Dentin after Selective Carious Tissue Removal

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ABSTRACT

Aim: This study aimed to evaluate the mineral deposition and microhardness within the retained caries-affected dentin (CAD) after excavation with Carisolv™ gel or rotary burs.

Materials and methods: Totally 20 extracted human carious molars, with caries extending into the middle third of dentin, were sectioned mesiodistally. The carious tissue in each hemi-section was excavated with gel or bur and restored with a mineral trioxide aggregate (MTA) base and resin composite. Five sound posterior teeth were hemi-sected to serve as a control. Twenty-five hemi-sections were stored in distilled water (DW) and the other 25 were stored in simulated body fluid (SBF). Each sample was scanned and tested using Raman spectroscopy and Knoop microhardness respectively, at the MTA–dentin interface at day 1 and 14. Scanning electron microscope (SEM) was used to assess the surface topography of dentin after excavation with gel/bur.

Results: The results show that the baseline mineral content and microhardness of dentin were significantly lower in Carisolv™-treated samples in comparison with that treated with burs ($p \leq 0.05$). However, there were comparable mineral levels in the two groups after 14 days' storage in SBF ($p \geq 0.05$), which showed statistically insignificant differences from the sound control ($p \geq 0.05$). Microhardness and mineral contents decreased significantly in the sound control after 14 days' storage in DW ($p \leq 0.05$). The SEM images showed partially open dentin tubules with less smear layer after Carisolv™ excavation compared with more occluded dentin tubules with an abundance of smear layer after bur excavation.

Conclusion: Although gel excavation retains more CAD in comparison with bur, remineralization of this remaining tissue is evident after 2 weeks.

Clinical significance: The use of Carisolv™ gel provides an alternative to rotary burs in terms of preserving the tooth structure and not hindering the remineralization potential of valuable tooth structure, a clinical advantage for minimally invasive dentistry.

Keywords: Carisolv, Dental caries, Microscopy, Remineralization.

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INTRODUCTION

The management of deep carious lesions in teeth with sensible pulps represents a challenge to clinicians. Traditionally, in order to provide a sound mineralized structural base for the restoration and to arrest further cariogenic activity within the lesion, complete removal of carious dentin (both infected and affected) was advised. However, the predictability of such a procedure is questionable as regards the increased risk of pulp exposure and devitalization.[1] Therefore, using contemporary minimally invasive management protocols, the preservation of pulp health and hard tissues is recommended when managing deep carious lesions.[2] Minimally invasive selective removal of carious tissue is the treatment of choice in deep cavitated dentin lesions in teeth with sensible/asymptomatic pulps,[2] reducing the risk of pulp exposure, preserving hard tissues CAD and arresting the cariogenic activity of bacteria.[3] However, concerns have been raised regarding the lower fracture strength and increased microleakage associated with partially excavated teeth compared with completely excavated teeth.[4] The annual failure rate for teeth with selective carious tissue removal was reported to be similar to or better than those with complete excavation. The latter group experiences most failures due to pulpitis and abscess formation.[5] Radiographic observations of the demineralized area under the restoration after partial carious tissue removal showed an increased radiodensity at follow-up periods.[6,7] The remineralization of the remaining demineralized dentin after selective carious tissue removal is a prerequisite to prevent further pulp irritation and to enhance the mechanical integrity of the tooth-restoration complex.

Remineralization of CAD can be achieved using bioactive materials, such as MTA. However, it is difficult clinically to differentiate CAD due to the nonselective nature of the traditional carious tissue excavation techniques. Chemomechanical systems, such as Carisolv™

gel (Rubicon Life Sciences, Sweden) prevent excessive removal of hard tissues by providing a self-limiting end point for carious tissue excavation.[8] The effects of Carisolv™ gel on the chemical, morphological, bacteriologic, and mechanical properties of the retained dentin surface have been investigated.[8-10] Results revealed a significant effect on the Vickers hardness of retained dentin, where Carisolv™ gel resulted in lower Vickers hardness number compared with rotary bur excavation.[9,10] Carisolv™ gel resulted in no obvious smear layer and open dentin tubules compared with a prominent smear layer and occluded dentinal tubules after carbon-steel bur excavation.[10,11] Carisolv™ gel excavation retains 50 µm more carious dentin than excavation with rotary burs.[12] Remineralization of this retained, demineralized CAD layer is of a clinical importance in maintaining the pulp health and mechanical integrity of the tooth-restoration complex by influencing the rate of ions exchange at this area.

Mineral trioxide aggregate has been used widely in dental applications including direct and indirect pulp capping in deep carious lesions. The MTA as a calcium source is a bioactive material forming apatite when it comes in contact with phosphate-containing fluids.[13] *In vitro* MTA/phosphate-containing fluid systems have been used to initiate apatite deposition in demineralized dentin with promising results.[14]

Many *in vitro* remineralization studies utilize artificially demineralized dentin samples. Clinically, it is important to explore whether using Carisolv™ gel for carious tissue excavation prevents remineralization of the residual carious dentin layer compared with rotary bur excavation, due to the initially low mineral levels in the residual CAD retained. This study aimed to investigate/monitor the mineral content and microhardness of CAD after excavation with Carisolv™ gel or rotary burs, using MTA as a repair material, SBF and DW as the storage media. The first null hypothesis was that there is no difference in mineral contents of CAD after excavation with Carisolv™ gel/rotary bur from that of sound dentin after 14 days' storage in SBF or DW. The second null hypothesis is that there is no difference in the microhardness of CAD after excavation with Carisolv™ gel/rotary bur from that of the sound dentin after 14 days' storage in SBF or DW.

MATERIALS AND METHODS

Sample Preparation

Twenty human extracted carious molars and five sound teeth were collected after obtaining the informed consent of the patients after National Research Ethics Service (NRES) Committee London-Riverside ethics approval (14/LO/0123). Teeth were stored in physiologic saline at 4°C for no more than 1 month and were imaged radiographically to verify the depth of the carious lesion. Teeth with lesions extending into/beyond the middle third of dentin radiographically were selected. Each carious tooth was cleaned with tap water for 2 minutes and then sectioned mesiodistally using a water-cooled diamond-impregnated circular saw (XL 12205, Benetec Ltd., London, UK). The teeth were divided into two groups of 20 halves; one was stored in DW and the other in SBF, both for 14 days. Each group was further divided into two subgroups (n = 10 hemi-sections for each subgroup): one was excavated with Carisolv™ gel and hand instruments, and the other 10 hemi-sections were excavated with rotary carbon-steel burs in a slow-speed handpiece. The five sound posterior teeth were sectioned mesiodistally and used as control subgroups (n = 5 halves/subgroup) and stored in either SBF or DW.

Rotary Burs

Lesion access was gained where necessary using a tungsten carbide bur ref. 878 to 2800 (Henry Schein, UK) in a high-speed air turbine handpiece Alegra TE-98 (W&H Dentalwerk Bürmoos GmbH, Salzburg, Austria) under water irrigation. Carbon-steel round burs (Ash instruments, Dentsply, Gloucester, UK) in a slow-speed handpiece WA56A (W&H Dentalwerk Bürmoos GmbH, Salzburg, Austria) (sizes 4 and 5; 5000–10000 rpm) were used to remove carious dentin with circular light brush strokes. The excavation endpoint was verified by using sharp dental explorer to verify the removal of soft "caries-infected" dentin, attempting to retain CAD which is sticky-scratchy in texture (clinical criteria).

Carisolv™ Gel

The equivalent hemi-section was excavated using chemomechanical Carisolv™ gel (RLS Global AB, Gothenburg, Sweden, batch no. E-1041-1), shown in Figure 1A. The gel is supplied as a twin-syringe, two-component system. The gel was auto-mixed in the correct proportions (Static Mixer, RLS Global AB, Gothenburg, Sweden) prior to the application on the carious dentin. Drops of gel were applied on the lesion after gaining the appropriate access with hand or rotary instruments at the level of the enamel–dentin junction. After 30 seconds, Carisolv™ hand instruments of the appropriate size were used to abrade away soft carious dentin. After the gel became cloudy, the cavity was rinsed with water and the procedure repeated until the gel remained clear; this helped to verify the excavation endpoint.

MTA Application

The MTA caps (Acteon, Pierre Rolland, Marignac, France) containing MTA powder and DW (Fig. 1B) were activated as per the manufacturer's instructions. The MTA was applied on the base of the prepared cavities and condensed

Figs 1A and B: Materials used in the study: (A) Carisolv™ gel is a twin-syringe system providing *in situ* mixing of sodium hypochlorite in one syringe and three amino acids (lysine, leucine, and glutamic acid) in a methylcellulose gel preparation in the other syringe. (B) MTA caps (Acteon, Pierre Rolland, Marignac, France) containing MTA powder and DW

with appropriate instruments. In order to prevent cement leakage across the cut surface, a glass slide was applied tightly against the sectioned tooth surface and sealed with sticky wax. The extra moisture in the MTA was removed with a cotton pellet or a paper point. The MTA was allowed to set initially for 1 hour in 100% humidity (placed on a wet cotton pellet) in 37°C before applying an etchant gel in the remaining cavity and rinsed with water and dried with air. A layer of Scotchbond™ Universal Bond dental adhesive (3M ESPE, USA) was applied and light cured for 20 seconds followed by a resin composite N'Durance (Septodont, Cedex, France) incremental restoration which was light cured for 40 seconds. After storage for 24 hours at 100% humidity and 37°C in either DW or SBF, the sectioned surfaces were polished using 1200, 2000, 2500, and 4000 grit carborundum papers for 1 minute each and cleaned in an ultrasonic bath with deionized water for 3 minutes after each polishing step before a final cleaning in ultrasonic bath for 5 minutes. Figure 2 shows an example of the study sample.

Remineralization Protocol

Simulated body fluid was prepared by dissolving 136.8 mM NaCl, 4.2 mM $NaHCO_3$, 3 mM KCl, 1 mM K_2HPO_4 $3H_2O$, 2.5 mM $CaCl_2$, and 0.5 mM $NaSO_4$ in deionized water, adding 3.08 mM sodium azide to prevent bacterial growth. The pH of the solution was controlled by alternate adding of 0.1 mM HCl and 0.1 mM Tris base until attaining a pH of 7.4. Each half was stored in a glass scintillation vial filled with 15 mL of SBF or DW for a period of 14 days. Each glass vial was capped to prevent evaporation of the solution and stored in an incubator at 37°C. The remineralization medium was changed every 3 days, with its pH monitored weekly.

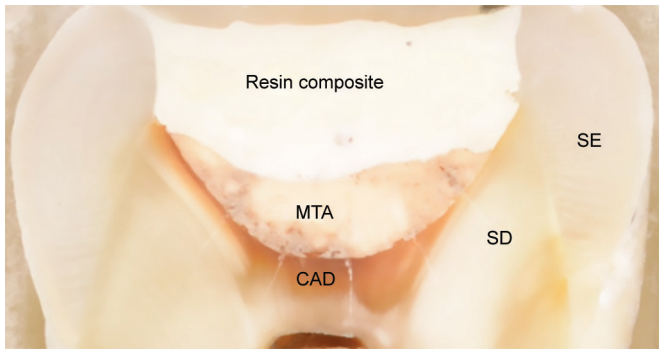


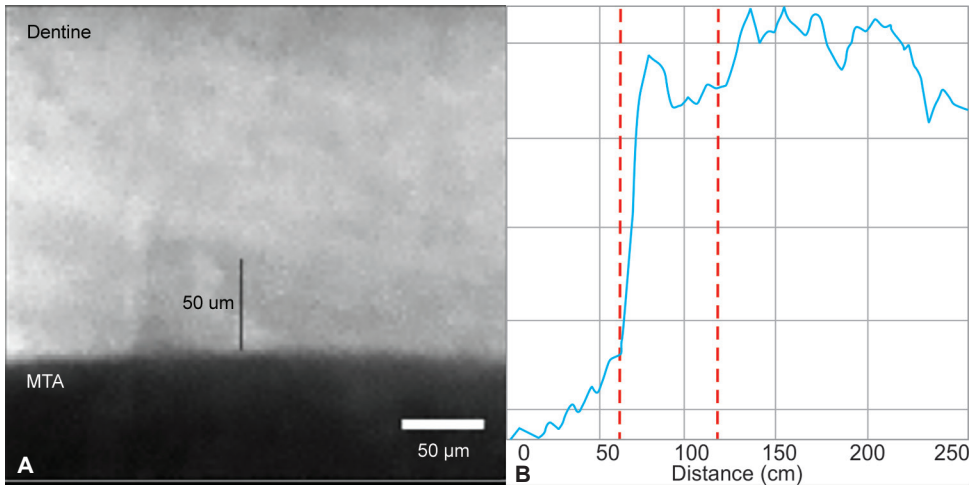
Fig. 2: Mineral trioxide aggregate applied on the base of the prepared cavities in Carisolv™-treated or rotary-treated hemi-sections followed by resin composite. SE: Sound enamel; SD: Sound dentin

Chemical and Mechanical Analysis

Raman Spectroscopy

The samples were examined using a Renishaw inVia Raman microscope (Renishaw Plc, Wotton-under-Edge, UK), as shown in Figure 3. The microscope was running in Streamline™ scanning mode to scan the sectioned surfaces with a 785-nm diode laser (100% laser power) focused using a 20/0.45 air objective. The signal was acquired using a 600 lines/mm diffraction grating

Fig. 3: Renishaw inVia Raman microscope (Renishaw Plc, Wotton-under-Edge, UK) used to monitor the MPI in treated hemi-sections



Figs 4A and B: (A) Representative gray-scale image of Raman phosphate peak intensity at 959 cm^{-1} including MTA and dentin areas of the scanned map with 50 µm measured area from the interface. (B) Depth profile of phosphate peak intensity along the scanned area illustrating phosphate peak intensity in the interfacial 50 µm

centered at 900 cm^{-1} and a charge-coupled device exposure time of 2 seconds. The microscope was calibrated using an internal silicon sample with a characteristic band at 520 cm^{-1}. Five Raman maps of the MTA/CAD interface were recorded for each sample at 24 hours and 14 days intervals. On day 14, an extra polishing step was performed before Raman scanning for 30 seconds with 4000 grit carborundum paper to remove any surface precipitates.

The Raman map started in the MTA and extended to the dentin to cover the surface area of 297 × 256.5 μm^2 of the interface and contained 10,450 spectra acquired with 2.7 µm resolution. For the analysis of Raman data, peak analysis was conducted by transferring the maps into an in-house-developed program (Raman Data Analysis 2 version 1.0) designed to fit the spectra and remove the cosmic rays, and then generate gray-scale images of phosphate peak intensity [mineral peak intensity (MPI)] at 959 cm^{-1} (PO_4^{3-} v1) across the sound and demineralized dentin areas. The average peak intensity of PO_4^{3-} in the first 50 µm in dentin from the MTA/dentin interface was calculated, as shown in Figure 4.

Knoop Microhardness

A Struers Duramin microhardness tester (Struers Ltd., Denmark) with a Knoop diamond indenter was used with a load of 25 gm applied for 10 seconds. The indentations were imaged with a 40/0.65 NA objective and the Knoop values were calculated using the manufacturer's software supplied. Two sets (n = 10/set) of measurements were recorded: the first set was recorded at 50 µm from MTA/dentin interface in dentin with 150 µm intervals. The second set of measurements recorded at 150 µm from MTA/dentin interface in dentin also recorded with 150 µm intervals. Measurements were then averaged to calculate the microhardness of each sample at 50 and 150 µm from interface and measurements were repeated on day 14.

Scanning Electron Microscope

One further tooth with a carious lesion extending half way into dentin was sectioned mesiodistally and each half was excavated with either chemomechanical carious tissue removal technique (Carisolv™ gel) or rotary burs. A SEM (FEI Co., Ltd., Cambridge, UK) was used to examine the ultrastructure of the excavated surfaces in each tooth (accelerating voltage of 3.5 and 10 kV, working distance of 10 mm, magnifications: 2,656 and 3,000 under high vacuum conditions).

Statistical Analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences statistical package (version 20; SPSS Inc., IBM, Chicago, Illinois, USA). Data were tested for normality using Q–Q plots and Shapiro–Wilk tests. For mineral contents, two factors were evaluated: the time (1 and 14 days) and the technique (Carisolv™ and rotary bur) in addition to the depth factor (50 and 150 µm) in microhardness analysis.

Paired sample t-test was used to compare between day 1 and 14 in each subgroup to assess the time factor. Independent sample t-test was used to compare between techniques (Carisolv™ and rotary bur) to assess technique factor and between different depths to assess the depth factor in microhardness. One-way analysis of variance (ANOVA) was used to compare between Carisolv™, rotary, and sound subgroups to assess the technique factor in general. Significant level was assumed at p = 0.05.

RESULTS

Mineral Content

The four internal vibration modes of phosphate ions (PO_4^{3-}) within dentin were observed as peaks at 433 cm^{-1} (symmetric bending vibrational mode—PO_4^{3-} ν2), 579 cm^{-1} (asymmetric bending vibrational mode—PO_4^{3-} ν4), 959 cm^{-1} (symmetric stretching vibrational mode—PO_4^{3-} ν1), and 1043 cm^{-1} (asymmetric stretching vibrational mode—PO_4^{3-} ν3). All peaks were observed within sound and demineralized dentin's spectra with no difference in their positions; the strongest peak along sound and demineralized dentin spectra was that of PO_4^{3-} ν1 at 959 cm^{-1}.

DW Group

Time factor: Paired sample t-test showed that the average phosphate peak intensity (MPI) within 50 µm from the interface decreased in each subgroup, in Carisolv™-treated samples (from 30.93 ± 2.92 to 26.83 ± 4.47 [mean ± standard error (SE)]) (p = 0.19) and rotary-treated samples (from 56.21 ± 2.85 to 39.22 ± 3.51 [mean ± SE]) (p = 0.001) and in sound control samples (from 146.3 ± 13.4 to 109.4 ± 18.2 [mean ± SE]) (p = 0.03) after 14 days.

Technique factor: At baseline measurements, there was a significant difference between Carisolv™-treated samples and rotary-treated samples at day 1 using independent sample t-test (p < 0.001), implying that less mineral phosphate content was present within the Carisolv™ gel excavated dentin initially. After 14 days, also there was a significantly higher mineral in rotary-treated samples compared with Carisolv™-treated samples (p = 0.03). The ANOVA showed that there were significant differences among the three subgroups: Carisolv™-treated, rotary-treated, and sound control samples at day 1 (p < 0.001) and 14 (p < 0.001). Table 1 shows the MPI of different subgroups in DW.

SBF Group

Time factor: Paired sample t-test showed that there was a significant increase in the MPI in each subgroup after 14 days except in sound control samples, in Carisolv™-treated samples (from 45.27 ± 3.20 to 109.79 ± 16.61 [mean ± SE] [p = 0.001]), rotary-treated samples (from 74.23 ± 10.40 to 132.64 ± 21.03 [mean ± SE]) (p = 0.01), and sound control samples (from 142.61 ± 8.94 to 152.46 ± 7.14 [mean ± SE] [p = 0.53]).

Technique factor: At baseline measurements, there was a significantly lower mineral in Carisolv™-treated samples compared with rotary-treated samples at day 1 using independent sample t-test (p = 0.01). After 14 days, there was 142.5 and 78.6% increase in the MPI in Carisolv™-treated samples and rotary-treated samples subgroups respectively; however, there was no significant difference between the Carisolv™-treated samples and rotary-treated samples subgroups after 14 days using independent sample t-test (p = 0.40). The ANOVA showed that there was a significant difference between Carisolv™-treated samples, rotary-treated samples, and sound control samples at 1 day (p < 0.001). However, there was no significant difference in the MPI between them at 14 days' interval (p = 0.44). Table 2 shows the MPI of different subgroups in SBF.

Knoop Microhardness

DW Group

Depth level: Independent sample t-tests showed that there were no significant differences in Knoop microhardness number (KHN) measurements between 50 and 150 µm points in Carisolv™-treated samples at both day 1 (p = 0.1) and day 14 (p = 0.1). Also there were no significant differences in KHN measurements between 50 and 150 µm points in rotary-treated samples at both day 1 and 14 (p = 0.09 and p = 0.09 respectively).

Time factor: Paired sample t-tests showed no significant difference in KHN in Carisolv™-treated samples and rotary-treated samples in each point at 50 and 150 µm after 14 days (p-values for Carisolv™-treated samples for 50 and 150 µm were p = 0.9 and p = 0.5 respectively. Rotary-treated sample p-values for 50 and 150 µm were p = 0.6 and p = 0.7 respectively). Sound control samples showed a significant decrease in KHN after 14 days (p < 0.001).

Table 1: Phosphate peak intensity average (mean ± standard error) within 50 µm from the interface in samples stored in DW and excavated with either Carisolv™ or rotary burs

DW group	*PO_4^{3-} V1 peak intensity average ± SE*	
Subgroup	*Day 1*	*Day 14*
Carisolv™-treated samples	30.9 ± 2.9[a]	26.8 ± 4.4
Rotary-treated samples	56.2 ± 2.8[a,b]	39.2 ± 3.5[b]
Sound control samples	146.3 ± 13.4[c]	109.4 ± 18.2[c]

Identical superscript letters indicate a statistically significant difference

Table 2: Phosphate peak intensity average (mean ± standard error) within 50 µm from the interface in samples stored in SBF and excavated either with Carisolv™ or with rotary burs

SBF	*PO_4^{3-} V1 peak intensity average ± SE*	
Subgroup	*Day 1*	*Day 14*
Carisolv™-treated samples	45.2 ± 3.2[a,b]	109.7 ± 16.6[a]
Rotary-treated samples	74.2 ± 10.4[b,c]	132.6 ± 21[c]
Sound control samples	142.6 ± 8.9	152.4 ± 7.1

Identical superscript letters indicate a statistically significant difference

Table 3: KHN (mean) at 50 and 150 µm from the interface between MTA and dentin in teeth stored in DW

	Knoop microhardness number			
	Day 1		*Day 14*	
Subgroup	*50 µm*	*150 µm*	*50 µm*	*150 µm*
Carisolv™-treated samples	18.6[a]	24.9[c]	16[b]	22.2[d]
Rotary-treated samples	40.6[a]	44.7[c]	38.8[b]	44.8[d]
Sound control samples	69.2[e]		27.9[e]	

Identical superscript letters indicate statistically significant difference

Table 4: KHN (mean) at 50 and 150 µm from the interface between MTA and dentin in teeth stored in SBF

	Knoop microhardness number			
	Day 1		*Day 14*	
Subgroup	*50 µm*	*150 µm*	*50 µm*	*150 µm*
Carisolv™-treated samples	18.8[a,b]	26.7[b,c]	27.8[a]	31.2[c]
Rotary-treated samples	31[e]	35.1[f]	44.3[e]	48.3[f]
Sound control samples	69		68.8	

Identical superscript letters indicate statistically significant difference

Technique factor: Independent sample t-test showed significant differences between Carisolv™-treated samples and rotary-treated samples at 50 and 150 µm points ($p < 0.001$ and $p < 0.001$ respectively) at day 1. Also, at day 14, there were significant differences between Carisolv™-treated samples and rotary-treated samples at 50 and 150 µm points ($p < 0.001$ and $p < 0.001$ respectively). The ANOVA showed significant differences between Carisolv™-treated samples, rotary-treated samples, and sound control samples at day 1 and 14 ($p < 0.001$ and $p < 0.001$ respectively). Table 3 shows the KHN of all subgroups in DW.

SBF Group

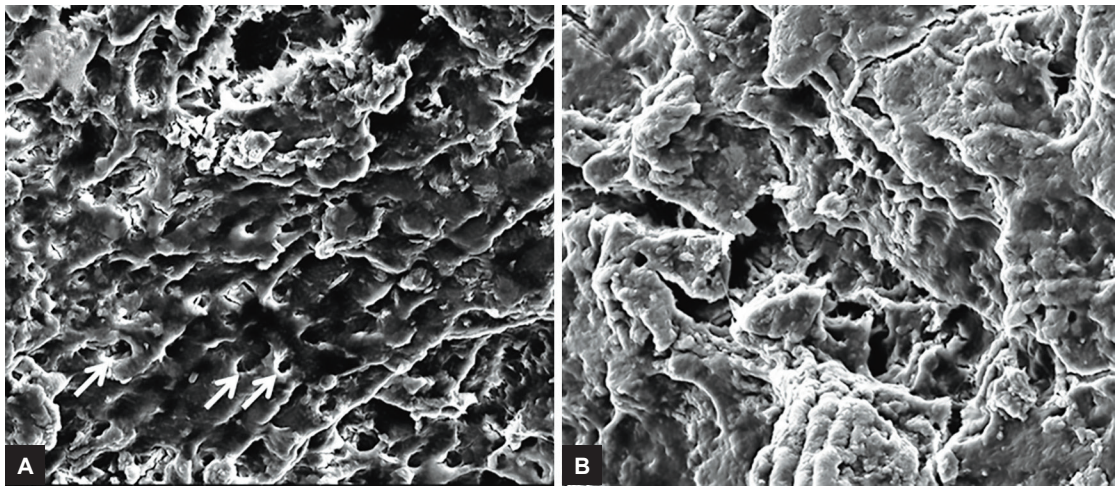
Depth level: Independent sample t-tests showed that there were significantly less KHN values at 50 µm compared with 150 µm in Carisolv™-treated samples at day 1 ($p = 0.002$), but there was no significant difference at day 14 ($p = 0.17$) between them. However, there was no significant difference in KHN between 50 and 150 µm in rotary-treated samples at day 1 and 14 ($p = 0.23$ and $p = 0.30$) respectively.

Time factor: Paired sample t-test showed a significant increase in KHN between day 1 and 14 in Carisolv™-treated samples and rotary-treated samples at 50 and 150 µm (p-values for Carisolv™-treated samples for 50 and 150 µm were $p < 0.001$ and $p = 0.04$ respectively; rotary-treated samples p-values for 50 and 150 µm were $p < 0.001$ and $p = 0.001$ respectively). Sound control samples showed no significant change in KHN between day 1 and 14 ($p = 0.67$).

Technique factor: Independent sample t-test showed significant differences between Carisolv™-treated samples and rotary-treated samples at 50 and 150 µm points ($p < 0.001$ and $p = 0.005$ respectively) at day 1. Also, at day 14, there were significant differences between Carisolv™-treated samples and rotary-treated samples at 50 and 150 µm points ($p < 0.001$ and $p < 0.001$ respectively). The ANOVA showed significant differences between 50 µm measurements of Carisolv™-treated samples, rotary-treated samples, and sound control samples at day 1 and 14 ($p < 0.001$ and $p < 0.001$ respectively). Table 4 shows the KHN of all subgroups in SBF.

Scanning Electron Microscope

The SEM images of carious dentin surface excavated with Carisolv™ gel showed that the openings of dentin tubules were partially occluded with smear layer. The SEM images of dentin surface excavated with rotary burs show complete occlusion of dentin tubules with smear layer as shown in Figure 5.



Figs 5A and B: SEM images of excavated dentin surface. (A) Carisolv™-excavated dentin surfaces at a magnification of 3000×; arrows point to open dentin tubule orifices. (B) Rotary burs excavated dentin surface show accumulation of smear layer occluding opening of smear layer occluding opening of dentin tubules

DISCUSSION

Raman spectroscopy has been used previously to characterize the different layers of sound and carious dentin lesions, and also to detect demineralization in dentin.[15] In addition, it has been proven to be effective in estimating the extent of mineral in enamel.[16] Also, it has been used to characterize the hybrid layer between dental adhesives and dentin.[17] Surface microhardness is one of the important properties of dental substrates. Its reduction indicates a dissolution, degradation, or demineralization in hard tissue structures.[18] Raman spectroscopy and Knoop microhardness were used conjointly in this study to monitor the remineralization in dentin. Raman and microhardness data at baseline indicated a significant reduction in the mineral content and hardness of dentin after excavation with Carisolv™ gel compared with rotary burs in the samples of both groups. This confirms that Carisolv™ gel retains more CAD compared with rotary burs in the cavity floor.[8,12] Rotary bur excavation led to a cavity endpoint located within more highly mineralized dentin which reflected the higher mineral and microhardness values observed at baseline measurements. Varied selectivity between the two excavation techniques produces cavities with different depths in CAD layer which has a gradual increase in hardness toward the sound inner dentin and pulp wall.[19]

After 2 weeks' storage in DW, mineral content in both rotary-treated samples and sound control samples decreased because of the pH of DW (pH = 6.5), which may have caused a potential dissolution of calcium and phosphate ions from tooth structure into the solution. The lack of bioavailable phosphate ions, a major constituent for mineral deposition, might lead to a reduction in microhardness and mineral content of dental hard tissues.[20] The transport of ions is considered a major factor in remineralization/demineralization processes.[21] However, in Carisolv™ gel-treated dentin, the overall content of mineral is low initially, which might not enhance the process of ion exchange with DW and result in an insignificant decrease in the mineral content compared with that in the rotary and control subgroups which both have a higher mineral content initially before storage. This can be explained by the fact that the amount of mineral present in approximately the first 50 µm of the lesion will influence the overall mineral profile after remineralization, possibly through influencing ion transport.[21]

Storage in SBF provided a source of phosphate ions in addition to calcium ions from the teeth and MTA, which resulted in a mineral deposition in both Carisolv™-treated samples and rotary-treated samples, and it was not significantly different from the level of mineral found in sound control samples after 14 days. It has been suggested that calcium ions from MTA interact with phosphate ions from SBF producing hydroxyapatite crystals in the MTA–dentin interface.[22] Others have suggested that calcium-deficient carbonated apatite crystallites form from the transformation of amorphous calcium phosphate, which is a key intermediate in biological apatite formation.[13] The ability to remineralize mineral-depleted CAD after selective/nonselective carious tissue excavation is evident from the results of this study. Control samples with higher mineral content did not acquire any further mineral deposition after 14 days in contrast to the intermediate mineral deposition found in samples excavated with rotary burs and high mineral deposition in samples excavated with Carisolv™ gel. This finding harmonizes with those from Kawasaki et al,[21] who found a significant effect of initial mineral level in the surface of the lesion on the subsequent mineral deposition rate and distribution. Therefore, the first hypothesis was accepted, as the mineral content in the retained dentin excavated with Carisolv™ gel did not significantly differ from that excavated with a rotary bur and that of sound dentin after 2 weeks. It also agreed with the *in vivo* results from Peters et al,[23] who found a significant increase in calcium and phosphate content in CAD underneath a calcium phosphate base material after 3 months, up to the level of 30 µm. The mineral content of treated CAD was within the range of healthy dentin, revealing the capacity of calcium phosphate base to promote remineralization of CAD *in vivo*.

The decrease in hardness of dentin in sound control samples after 14 days may be attributed to the absence of both phosphate (from SBF) and calcium (from MTA) ions in the DW solution. Dissolution of ions from tooth structure into DW could lead to a decrease in hardness of control samples. In contrast, storage in SBF led to ion deposition which increased the microhardness of CAD after 14 days. Although the hardness of rotary-treated samples was significantly higher than that of Carisolv™-treated samples after 1 day storage in SBF, in both subgroups, it did not reach that of sound control samples after 14 days. Although the mineral content of CAD after excavation with both techniques was comparable to that of sound dentin after 2 weeks, CAD did not improve its hardness compared with that of sound dentin. Other studies showed similar results; there was a nonlinear correlation between mineral density and mechanical properties of wet remineralized dentin, especially in the absence of intratubular remineralization.[24] In addition, the hardness of CAD is lower than sound dentin, because of a decrease in the number and size of apatite crystals in intertubular dentin in CAD due to demineralization.[25] The second hypothesis was rejected because microhardness of dentin excavated with Carisolv™ gel did not reach

the level of microhardness of dentin excavated with a rotary bur or that of sound dentin after 14 days' storage. Others suggest that whitlockite rather than apatite is being precipitated.[26] However, in the present study, the deposition of hydroxyapatite in dentin is specific, as the characteristic Raman peaks of other possible minerals, such as octa-calcium phosphate, di-calcium phosphate dihydrate, tricalcium phosphate, or amorphous calcium phosphate show the PO_4^{3-} v1 band at different wave number shifts (cm^{-1}),[27] than that was found in the sample analysis in this study.

In the present study, the composition of remineralization solution had a significant effect on mineral gain and microhardness in dentin and this agrees with other studies.[20,28] The factors that influence the bioavailability of calcium and phosphate ions play a substantial role in promoting ion precipitation in the demineralized CAD. The saturated level of these ions in SBF promotes the precipitation of ions clusters in the voids of the demineralized tissue. Naturally, saliva provides bioavailable calcium and phosphate ions to remineralize the demineralized enamel.[29] Although fluoride plays an important role in enamel remineralization by controlling calcium and phosphate ion uptake, dentin remineralization is difficult to achieve in the presence of fluoride.[30] Surface examination revealed remineralization on enamel surface, but not on dentin surface with the same remineralization parameters.[31] Extracellular matrix phosphoproteins, such as dentin phosphoprotein can regulate growth and inhibition of apatite nucleation in collagenous tissue; it binds to collagen surface and lowers the interfacial energy for hydroxyapatite nucleation. Others inhibit crystal growth by producing an electrostatic repulsion of inorganic phosphate ions once the protein is adsorbed into the crystal surface.[32] In dentin remineralization, the availability of these ions can be compromised by the presence of translucent dentin at the advancing front of the lesion, which will minimize saturated fluids levels to enhance the remineralization process of dentin. In addition, the presence of individual plasma protein fractions and several different types of bacteria can influence the permeability of fluids across the dentin.[33]

The dissimilar SEM patterns of the dentin surfaces obtained in the two techniques might have an effect on the mineral precipitation, hardness, and dentin permeability. It has been found that microleakage decreased in teeth filled with resin composite after treatment with Carisolv™ gel due to smear layer-free irregular surface that can enhance adhesion to adhesive restorative materials as confirmed by SEM.[34] However, smear layer-free/-rich (etched/nonetched) CAD underneath the calcium phosphate base resulted in no difference in mineral precipitation after *in vivo* 3 months' service.[23] Regardless of open dentin tubules in Carisolv™ gel-treated dentin, mineral levels were similar to those found in rotary bur-treated dentin after 2 weeks SBF storage but with a higher remineralization percentage.

The rationale of using MTA in this study (pH 11.7) was to simulate the clinical situation in cases of deep caries in patients that need minimally invasive carious tissue removal strategies, where the retained dentin after carious tissue removal should be sealed immediately with a nonirritating and sealing material.[35] The documented sealing ability and biocompatibility of MTA could make it a suitable choice to prevent an inflammatory response in dental pulp after excavation of carious tissue in deep lesions and a carious dentin remineralization promoter.[36] In addition, the roles of MTA in the initiation of release of dentin bioactive components (noncollagenous proteins, glycosaminoglycans, and transforming growth factor β1) have been proven *in vitro* and clinically these components have the potential to regulate dentin repair and regeneration which include remineralization.[37,38] The presence of MTA as a source of calcium and hydroxyl ions and as an initiator of dentin bioactive components release could have a reservoir effect on the samples stored in DW. This could create a chemical equilibrium and raise pH above 7,[39] which could prevent further dissolution of ions from tooth structure into DW. This probably led to a nonsignificant decrease in hardness of samples excavated with Carisolv™ or rotary bur compared with the significant drop in the control subgroup, which has no MTA.

Limitations of the study include the variability of the naturally demineralized carious dentin samples compared with artificially demineralized dentin used in many previous studies. Although many studies have been conducted on remineralization of artificially demineralized dentin,[40] remineralization of natural carious dentin represents the most realistic model of the clinical condition *per se*. The inability to remove MTA from the excavated surfaces without leaving remnants limited the chance to examine the excavated surfaces after 14 days of storage under SEM.

The repair potential of mineral-deficient carious dentin after minimally invasive carious tissue removal is important, as it helps to increase resistance to mechanical forces, delay bacterial penetration, and prevent caries recurrence, in addition to the advantage of tooth preservation that is associated with minimal carious tissue removal approach.

CONCLUSION

Within the limitations of the present study, it was found that remineralization of natural CAD is possible after excavation either with Carisolv™ gel or rotary burs confirming the selective minimally invasive nature of

Carisolv™ gel in comparison with rotary burs. Also, there is a significant influence of the type of storage solution on the remineralization of dentin *in vitro*.

CLINICAL SIGNIFICANCE

The use of Carisolv™ gel provides an alternative to rotary burs in terms of preserving tooth structure and ability to remineralize valuable tooth structure, a clinical advantage for minimally invasive dentistry.

REFERENCES

1. Thompson V, Craig RG, Curro FA, Green WS, Ship JA. Treatment of deep carious lesions by complete excavation or partial removal: a critical review. JADA 2008 Jun;139(6):705-712.
2. Schwendicke F, Frencken JE, Bjørndal L, Maltz M, Manton DJ, Ricketts D, Van Landuyt K, Banerjee A, Campus G, Doméjean S, et al. Managing carious lesions consensus recommendations on carious tissue removal. Adv Dent Res 2016 May;28(2): 58-67.
3. Ricketts D, Lamont T, Innes NP, Kidd E, Clarkson JE. Operative caries management in adults and children. Cochrane Database Syst Rev 2013 Mar;3:CD003808.
4. Hevinga MA, Opdam NJ, Frencken JE, Truin GJ, Huysmans MC. Does incomplete caries removal reduce strength of restored teeth? J Dent Res 2010 Nov;89(11):1270-1275.
5. Schwendicke F, Meyer-Lueckel H, Dörfer C, Paris S. Failure of incompletely excavated teeth—a systematic review. J Dent 2013 Jul;41(7):569-580.
6. Maltz M, de Oliveira EF, Fontanella V, Bianchi R. A clinical, microbiologic, and radiographic study of deep caries lesions after incomplete caries removal. Quintessence Int 2002 Feb;33(2):151-159.
7. Alves LS, Fontanella V, Damo AC, Ferreira de Oliveira E, Maltz M. Qualitative and quantitative radiographic assessment of sealed carious dentin: a 10-year prospective study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010 Jan;109(1):135-141.
8. Krause F, Braun A, Lotz G, Kneist S, Jepsen S, Eberhard J. Evaluation of selective caries removal in deciduous teeth by a fluorescence feedback-controlled Er:YAG laser *in vivo*. Clin Oral Investig 2008 Sep;12(3):209-215.
9. Sakoolnamarka R, Burrow MF, Swain M, Tyas MJ. Microhardness and Ca:P ratio of carious and Carisolv™ treated caries-affected dentine using an ultra-micro-indentation system and energy dispersive analysis of x-rays—a pilot study. Aust Dent J 2005 Apr;50(4):246-250.
10. Hamama HH, Yiu CK, Burrow MF, King NM. Chemical, morphological and microhardness changes of dentine after chemomechanical caries removal. Aust Dent J 2013 Sep;58(3):283-292.
11. Banerjee A, Kidd EA, Watson TF. Scanning electron microscopic observations of human dentine after mechanical caries excavation. J Dent 2000 Mar;28(3):179-186.
12. Splieth C, Rosin M, Gellissen B. Determination of residual dentine caries after conventional mechanical and chemomechanical caries removal with Carisolv. Clin Oral Investig 2001 Dec;5(4):250-253.
13. Tay FR, Pashley DH, Rueggeberg FA, Loushine RJ, Weller RN. Calcium phosphate phase transformation produced by the interaction of the Portland cement component of white mineral trioxide aggregate with a phosphate-containing fluid. J Endod 2007 Nov;33(11):1347-1351.
14. Reyes-Carmona JF, Felippe MS, Felippe WT. The biomineralization ability of mineral trioxide aggregate and portland cement on dentin enhances the push-out strength. J Endod 2010 Feb;36(2):286-291.
15. Almahdy A, Downey FC, Sauro S, Cook RJ, Sherriff M, Richards D, Watson TF, Banerjee A, Festy F. Microbiochemical analysis of carious dentine using Raman and fluorescence spectroscopy. Caries Res 2012 Jun;46(5):432-440.
16. Milia E, Pinna R, Castelli G, Bortone A, Marceddu S, Garcia-Godoy F, Gallina G. TEM morphological characterization of a one-step self-etching system applied clinically to human caries-affected dentin and deep sound dentin. Am J Dent 2012 Dec;25(6):321-326.
17. Hashimoto M, Ohno H, Kaga M, Sano H, Endo K, Oguchi H. The extent to which resin can infiltrate dentin by acetone-based adhesives. J Dent Res 2002 Jan;81(1):74-78.
18. Salem-Milani A, Zand V, Asghari-Jafarabadi M, Zakeri-Milani P, Banifatemeh A. The effect of protocol for disinfection of extracted teeth recommended by center for disease control (CDC) on microhardness of enamel and dentin. J Clin Exp Dent 2015 Dec;7(5):e552-e556.
19. Ogawa K, Yamashita Y, Ichijo T, Fusayama T. The ultrastructure and hardness of the transparent of human carious dentin. J Dent Res 1983 Jan;62(1):7-10.
20. Habelitz S, Marshall GW Jr, Balooch M, Marshall SJ. Nanoindentation and storage of teeth. J Biomech 2002 Jul;35(7): 995-998.
21. Kawasaki K, Ruben J, Tsuda H, Huysmans MC, Takagi O. Relationship between mineral distributions in dentine lesions and subsequent remineralization *in vitro*. Caries Res 2000 Sep-Oct;34(5):395-403.
22. Sarkar N, Caicedo R, Ritwik P, Moiseyeva R, Kawashima I. Physicochemical basis of the biologic properties of mineral trioxide aggregate. J Endod 2005 Feb;31(2):97-100.
23. Peters MC, Bresciani E, Barata TJ, Fagundes TC, Navarro RL, Navarro MF, Dickens SH. *In vivo* dentin remineralization by calcium-phosphate cement. J Dent Res 2010 Mar;89(3): 286-291.
24. Kinney JH, Habelitz S, Marshall SJ, Marshall GW. The importance of intrafibrillar mineralization of collagen on the mechanical properties of dentin. J Dent Res 2003 Dec;82(12):957-961.
25. Fusayama, T. Simple pain-free adhesive restorative system by minimal reduction and total etching. Tokyo: Ishiyaku EuroAmerica, Incorporated; 1993.
26. Fusayama T. Intratubular crystal deposition and remineralization of carious dentin. J Biol Buccale 1991 Sep;19(3):255-262.
27. Koutsopoulos S. Synthesis and characterization of hydroxyapatite crystals: a review study on the analytical methods. J Biomed Mater Res 2002 Dec;62(4):600-612.
28. Schwendicke F, Al-Abdi A, Meyer-Luckel H, Paris S. Pulpal remineralisation of artificial residual caries lesions *in vitro*. Caries Res 2015 Oct;49(6):591-594.
29. Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New approaches to enhanced remineralization of tooth enamel. J Dent Res 2010 Nov;89(11):1187-1197.
30. Damen JJ, Buijs MJ, ten Cate JM. Fluoride-dependent formation of mineralized layers in bovine dentin during demineralization *in vitro*. Caries Res 1998 Nov-Dec;32(6):435-440.

31. Fan Y, Sun Z, Moradian-Oldak J. Controlled remineralization of enamel in the presence of amelogenin and fluoride. Biomaterials 2009 Feb;30(4):478-483.
32. George A, Veis A. Phosphorylated proteins and control over apatite nucleation, crystal growth, and inhibition. Chem Rev 2008 Nov;108(11):4670-4693.
33. Pashley DH, Nelson R, Kepler EE. The effects of plasma and salivary constituents on dentin permeability. J Dent Res 1982 Aug;61(8):978-981.
34. Yamada Y, Kimura Y, Hossain M, Kinoshita JI, Shimizu Y, Matsumoto K. Caries removal with Carisolv system: criteria evaluation and microleakage test. J Clin Pediatr Dent 2005 Winter;30(2):121-126.
35. Langeland, K. Tissue changes in the dental pulp: an experimental study. Oslo: Oslo University Press; 1957.
36. Prati C, Gandolfi MG. Calcium silicate bioactive cements: biological perspectives and clinical applications. Dent Mater 2015 Apr;31(4):351-370.
37. Tomson PL, Grover LM, Lumley PJ, Sloan AJ, Smith AJ, Cooper PR. Dissolution of bio-active dentine matrix components by mineral trioxide aggregate. J Dent 2007 Aug;35(8):636-642.
38. Ferracane JL, Cooper PR, Smith AJ. Can interaction of materials with the dentin-pulp complex contribute to dentin regeneration? Odontology 2010 Feb;98(1):2-14.
39. Duarte MA, Demarchi AC, Yamashita JC, Kuga MC, de Campos Fraga S. pH and calcium ion release of 2 root-end filling materials. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003 Mar;95(3):345-347.
40. Tay FR, Pashley DH. Biomimetic remineralization of resin-bonded acid-etched dentin. J Dent Res 2009 Aug;88(8):719-724.