Analysis of Microstructural Changes of Sound, Demineralized and Denatured Dentin on Application of Carisolv – An In Vitro Study

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Abstract

BACKGROUND AND OBJECTIVES: The purpose of this study was to analyze the microstructural changes caused by Carisolv gel on sound, demineralized, and denatured dentin.

METHOD: A total of 48 freshly extracted human premolars and molars were collected and divided into three main groups. Group I contained 12 teeth with fractured dentinal surface, which were further divided into two subgroups IA and IB, which contained 6 teeth in each. Group II contained 18 teeth with dentinal surface demineralized superficially by phosphoric acid etching and were divided into three subgroups IIA, IIB and IIC, which contained 6 teeth in each. Group III contained 18 teeth, where dentinal surface was denatured using lactic acid and collagenase pretreatment, and was divided into three subgroups IIIA, IIIB and IIIC. 6 teeth from each group were exposed to freshly mixed Carisolv gel and 6 teeth exposed to 0.25% sodium hypochlorite. No additional mechanical action was exerted during the 20 minutes exposure of specimen to the Carisolv gel. Specimens were evaluated by scanning electron microscope. Data was analyzed by using paired t-test for intragroup comparisons and ANOVA for intergroup comparisons followed by Post-hoc Tukey’s test for group wise comparisons.

RESULTS: Scanning electron microscope evaluation did not indicate any microstructural changes of the dentinal surfaces in Group I or Group II, due to the 20 minutes Carisolv gel. Denatured dentin in Group III was partially removed within a 20 minutes period of chemical action of the Carisolv gel leaving only a 20.2-21.1µm thick layer of residual denatured dentin on the specimen’s surface. In contrast, 0.25% sodium hypochlorite treatment completely dissolved the demineralized as well as denatured dentin layer within 20 minutes.

CONCLUSION: From this in vitro study, it can be concluded that Carisolv gel does not affect sound fractured dentin, and does not dissolve demineralized dentin and has a limited potential to chemically dissolve denatured dentin.

Keywords: Carisolv gel, demineralized dentin, denatured dentin, lactic acid, phosphoric acid, and sound dentin.
Introduction

Over time, modern dentistry has evolved to a minimally invasive approach, in which caries is managed as an infectious disease, deferring operative intervention as long as possible. The focus is on maximum conservation of demineralized, non cavited enamel and dentin. It is now well recognized that it is possible to arrest and even reverse the mineral loss associated with caries at an early stage. Enamel and dentin demineralization is not a continuous, irreversible process. Through a series of demineralization and remineralization cycles, the tooth alternatively loses and gains calcium and phosphate ions, depending on the microenvironment [1].

Carious dentin consists of two layers, the superficial first layer, characterized by extensive decalcification, degenerated collagen fibers and negative odontoblastic process that is physiologically unrecalciifiable. The underlying second layer is characterized by intermediate decalcification, sound collagen fibers and living odontoblastic process that is physiologically recalciifiable [2]. Conventional drilling results in a rapid and excessive removal of uninfected dentin and may cause harmful thermal and pressure effects to the pulp. Thus, there is a need to remove caries with a less painful and more tissue preservation manner, like demineralized dentin, which results in preservation of the tooth structure [3]. Minimally-invasive dentistry comprises biologically oriented procedures. The teeth are treated with precision and caution in order to last as long as possible, avoid post-operative complications and fulfill the demands of present day patients [1].

Chemo-mechanical caries removal brings us close to minimally-invasive procedures as possible. The method involves the chemical softening of carious dentin followed by its removal by gentle excavation. The reagent generated by mixing amino acids with sodium hypochlorite, N-monochloro amino acids are formed which selectively degrade the demineralized collagen in carious dentin [3,4]. The purpose of this study was to analyze the microstructural changes caused by chemo-mechanical agent i.e. Carisolv gel on sound, demineralized, and denatured dentin.

Materials and methods

A total of 48 freshly extracted human premolars and molars were used in the study. These teeth were stored in phosphate buffered saline (PBS). The fractured dentinal surface was produced, by breaking away the occlusal third of the crowns using a chisel after preparation of a circular groove across the enamel up the dentin of the teeth, thereby 48 specimens with fractured dentinal surface were produced.

They were divided into 3 main groups, Group I contained 12 teeth with fractured dentinal surface, which was further divided...
into two subgroups IA and IB, which contained 6 teeth in each. Group II contained 18 teeth with dentinal surface demineralized superficially by phosphoric acid etching, and was divided into three subgroups IIA, IIB and IIC, which contained 6 teeth in each. Group III contained 18 teeth, where dentinal surface was denatured by stepwise incubation in 0.1 M lactic acid (pH 4.0 in PBS) for 24 hrs and subsequently in collagenase type D and were divided into three subgroups IIIA, IIIB and IIIC, which contained 6 teeth in each.

From each group 6 teeth were exposed to freshly mixed Carisolv gel (Medi team) and 6 teeth were exposed to 0.25% sodium hypochlorite. No additional mechanical action was exerted during the 20 minutes exposure of specimens to the Carisolv gel and subsequently specimens were rinsed in water. For control purpose, 6 fractured, 6 demineralized and 6 denatured dentinal surfaces were left unexposed in Carisolv gel or 0.25% sodium hypochlorite.

The specimens were stored in water for 24 hours at 37° C and then, the study samples were vertically sectioned with a hard tissue microtome (Lecia SP 1600) under water lubrication, through the superficial fractured dentin, into approximately 1mm thick slabs.

The specimens were then coated with a thin layer of gold and observed under Scanning Electron Microscope at 1000x magnification. One reading was carried out for each of 12 specimens in Group I and 18 specimens in Group II and III. Data was analyzed by using paired t-test for intragroup comparisons and ANOVA for intergroup comparisons followed by Post-hoc Tukey’s test for group wise comparisons.

Results
There was no statistically significant difference between the Subgroup IA and Subgroup IB [Table 1]. Subgroup IIA and IIB showed no significant difference. However, there was significant difference between subgroup IIA and IIC, where subgroup IIC had less thickness of demineralized dentin. There was also significant difference between subgroup IIB and IIC, where subgroup IIC had less thickness of demineralized dentin [Table 2]. On statistical analysis, it showed significant difference between subgroup IIIA, IIIB and IIIC, where the thickness of denatured dentin was more in subgroup IIIA compared to subgroup IIIB and IIIC. Subgroup IIIB showed less thickness of denatured dentin compared to subgroup IIIA, and more thickness than subgroup IIIC. Subgroup IIIC showed less thickness of denatured dentin compared to subgroup IIIA and IIIB [Table 3].

Scanning electron microscope (SEM) evaluation did not indicate any microstructural changes of the fractured dentinal surface in group I [Fig 1, 2] and demineralized dentinal surfaces in group II due to the 20 minutes Carisolv gel [Fig 3, 4]. Denatured dentin was partially removed.
within a 20 minutes period of chemical action of the Carisolv gel leaving only a 20.2-21.1µm thick layer of residual denatured dentin on the specimen’s surface [Fig 6, 7]. In contrast, 0.25% sodium hypochlorite treatment completely dissolved the demineralized as well as denatured dentin layer within 20 minutes. [Fig 5, 8] However, the scanning electron microscope showed that under the experimental conditions chosen in this in vitro study the Carisolv gel did not completely remove, but selectively dissolved the denatured dentin.

**Discussion**

Current dental restorative concepts are characterized by an increased effort towards a less invasive treatment of carious lesions. Minimally invasive cavity preparation technique are intended to preserve as much sound enamel and dentin as possible during treatment of carious lesions, because it appears that only soft, wet dentin is heavily infected with bacteria, any technique that effectively removes such infected dentine should be adequate to halt the carious process, then the cavity is sealed. Dentine consists of mineral (70% by wt), water (10% by wt) and an organic matrix (20% by wt). Of this organic matrix, 18% is collagen and 2% non-collagenous compounds including chondroitin sulphate, other proteoglycans and phosphophoryns. Collagen is an unusual protein containing large amounts of proline and one third of the amino acid content is glycine. The polypeptide chains are coiled into triple helices which are known as tropocollagen units; these tropocollagen units then orientate side by side to form a fibril. Covalent bonds between the polypeptide chains and between the tropocollagen units form cross links and give the collagen fibrils stability, in dentine the fibrils are in the form of a dense meshwork which becomes mineralized [3,4].

When caries occurs, acids produced by plaque bacteria by anaerobic fermentation of carbohydrate initially cause solubility of the mineral in enamel. As the process progresses, dentinal tubules provide access for penetrating acids and subsequent invasion by bacteria which results in a decrease in pH and causes further acid attack and demineralization. When the organic matrix has been demineralised, the collagen and other matrix components are then susceptible for enzymatic degradation, mainly by bacterial proteases and other hydrolases [3]. With respect to collagen degradation, two zones can usually be distinguished within a lesion. There is an inner layer which is partially demineralised and can be remineralised and in which the collagen fibrils are still intact, and there is an outer layer where the collagen fibrils are partially degraded and cannot be remineralised. A chemo-mechanical caries removal reagent must be able to cause further degradation of this partially degraded collagen, by cleavage of the polypeptide chains in the triple helix and by hydrolyzing the cross linkages [1,3,5].
Originally it was thought that the procedure involved is the chlorination of partially degraded collagen in the carious lesion and the conversion of hydroxyl proline to pyrrole-2-carboxylic acid. Recent work suggests that cleavage by oxidation of glycine residues could also be involved. This causes disruption of collagen fibrils which become more friable and then can be removed. Caridex system initially proved to be quite popular, but large volumes of solution were needed (200-500 ml) and the procedure was slow. Only certain cavities were suitable for treatment by the technique and because of the time involvement of 10-15 minutes and limited use, popularity in the US waned. An attempt was made to improve the reagent by the addition of urea, which normally denatures proteins by breaking down hydrogen bonding thereby making them more soluble. Although urea itself was no better than a saline control as a chemomechanical caries removal reagent, addition of urea to N-monochloro-DL-2-aminobutyrate improved the efficacy of the formulation [3]. During this time, Medi Team in Sweden continued to work on the system and the latest chemomechanical caries removal reagent known as Carisolv was launched.

The carisolv gel is considered to soften the carious altered dentin, but preserves the sound dentin and does not affect the surface topography of healthy dentin to more than a negligible degree [6]. In our present study fractured dentinal surface were chosen to investigate the effect of Carisolv gel, because they were more susceptible to changes caused by chemical agents than smear layer covering the dentinal surface.

A 0.25% concentration of sodium hypochlorite was chosen in the present study, because this is the concentration of sodium hypochlorite present in the Carisolv gel after mixing of both components. In contrast to Carisolv gel, the 0.25% sodium hypochlorite solution effectively removed the layer of demineralized collagen fibers from the dentinal surface. The difference between the action of Carisolv gel containing 0.25% sodium hypochlorite and the pure 0.25% sodium hypochlorite solution could be explained by the amino acids added to Carisolv gel. The amino acids might react with sodium hypochlorite, thus reducing the organic tissue solving properties of the sodium hypochlorite in Carisolv gel [7]. In our study, it was seen that the sound fractured dentin did not reveal any significant microstructural changes, even after 20-min exposure to Carisolv gel.

A 20 minute exposure time was chosen in the present study, since the manufacture of the Carisolv gel stated that the activity of the freshly mixed gel is limited to a 20-min period after mixing. Thus, from a microstructural point of view, application of Carisolv gel to healthy dentin gives no cause for concern [Fig 2]. These results are in
acCORDANCE WITH THE STUDY CONDUCTED BY GWINNETT AJ ET AL AND KUROSAKI T AT SIMILAR RESULTS REGARDING THE EFFECT OF CARIDEX WHICH DID NOT AFFECT THE SOUND DENTINE [8,9].

IT WAS EVIDENT FROM THE SCANNING ELECTRON MICROSCOPE THAT ARTIFICIALLY DEMINERALIZED DENTIN WAS NOT DISSOLVED DURING THE 20 MINUTES EXPOSURE TO FRESHLY MIXED CARISOLV GEL. PHOSPHORIC ACID PRETREATMENT CAUSED DEMINERALIZATION OF THE DENTINAL SURFACE LEAVING A 21.89-22.5 µM THICK LAYER OF Densely PACKED COLLAGENIC FIBERS [FIG 3]. THIS LAYER OF DEMINERALIZED DENTIN WAS NOT ALTERED IN MICROMORPHOLOGICAL PROPERTY OR IN THICKNESS DUE TO THE 20 MINUTES EXPOSURE IN CARISOLV GEL [FIG 4]. HOWEVER, A 20 MINUTES EXPOSURE OF THE PHOSPHORIC ACID PRE- TREATED DENTIN IN 0.25% SODIUM HYPOCHLORITE COMPLETELY REMOVED THE DEMINERALIZED COLLAGENIC FIBRIL NETWORK FROM THE DENTINAL SURFACE [FIG 5].

THIS RESULTS WAS IN ACCORDANCE WITH THE STUDY CONDUCTED BY TILL DAMMASCHKE ET AL WHICH STATED THAT THE REACTIVITY OF CARISOLV RADICALS APPEARS TO BE ATTENUATED TO A LEVEL SUFFICIENT FOR DECOMPOSITION OF CELLULAR COMPONENTS (E.G. MEMBRANES) BUT NOT FOR DECOMPOSITION OF EXTRACELLULAR MATRIX COMPONENTS (E.G. COLLAGENOUS FIBRILS), AND OFFERS LESS REACTIVE GROUPS FOR THE CARISOLV AGENTS UNLESS THEY HAVE PREVIOUSLY BEEN DAMAGED RESULTING IN THE EXPOSURE OF INTERNAL REACTIVE GROUPS AND ALSO IN THE GENERATION OF NEW REACTIVE GROUPS. HENCE THE ORGANIC MATRIX IS PROTECTED BY THE MINERAL CRYSTALS ENVELOPING THE DENTIN FIBRILS AND THE CRYSTAL THEMSELVES CANNOT BE ATTACKED BY THE RADICALS [5].

A STUDY CONDUCTED BY KUBOKI Y ET AL AND OHGUSHI K ET AL REPORTED PREVIOUSLY THAT THE INNER PART OF THE CARIOS LAYER IN DENTIN IS ONLY SLIGHTLY DEMINERALIZED AND CONTAINS AN INTACT ORGANIC MATRIX WITH UNALTERED COLLAGENIC FIBRES [2,10,11]. A STUDY CONDUCTED BY KATO S AND FUSAyAMA SHOWED, THIS COLLAGEN FRAMEWORK HAS THE POTENTIAL FOR REMINERALIZATION. THEREFORE, IT MIGHT BE SUGGESTED THAT TREATMENT WITH CARISOLV GEL POSSIBLY PRESERVES THE INTACT COLLAGENIC MATRIX OF THE DENTIN WHICH COULD BE RECALCIFIED [1].

It should be kept in mind when interpreting the present results that this in vitro investigation focused on the chemical action of Carisolv gel on sound, demineralized and denatured dentinal surfaces rather than on caries removal with the Carisolv method which involves additional mechanical force application.

Conclusion
Within the limitations of this in-vitro study the following conclusions can be drawn from the results of this study that Carisolv gel did not affect sound fractured dentin and did not dissolve demineralized dentin. Carisolv gel was effective in removing denatured dentin which had a limited potential to chemically dissolve denatured dentin. In an environment, in which “extraction is the rule rather than an exception” as in the developing countries, unconventional tooth preserving approaches such as the atraumatic restorative treatment have an opportunity to evolve. Application of this approach, which does not rely on electricity or expensive dental equipment, makes it possible to provide a cost effective treatment for large population.

References

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Figure 1: SEM showing the thickness of sound dentin.

Figure 2: SEM showing the thickness of dentin exposed to carisolv gel.

Figure 3: SEM showing the thickness of demineralized dentin.

Figure 4: SEM showing the thickness of demineralized dentin exposed to carisolv gel.

Figure 5: SEM showing the thickness of demineralized dentin exposed to 0.25% sodium hypochlorite.

Figure 6: SEM showing thickness of denatured dentin.
Table 1: The Values Obtained In Terms Of Means For Sound Dentin And Sound Dentin Exposed To Carisolv

<table>
<thead>
<tr>
<th>Group I</th>
<th>Mean ± S.D. (μm)</th>
<th>Range (μm)</th>
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<tbody>
<tr>
<td>Subgroup IA (sound dentin)</td>
<td>88.65 ± 0.51</td>
<td>87.8-89.1</td>
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<tr>
<td>Subgroup IB (sound dentin + Carisolv)</td>
<td>88.55 ± 0.63</td>
<td>87.4-89.1</td>
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<td>Unpaired t-test</td>
<td>p= 0.77, not significant</td>
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</tr>
<tr>
<td>Group II</td>
<td>Mean ± S.D. (μm)</td>
<td>Range (μm)</td>
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<tr>
<td>Subgroup IIA (demineralized dentin)</td>
<td>22.2 ± 0.28</td>
<td>21.89-22.5</td>
</tr>
<tr>
<td>Subgroup IIB (demineralized dentin + Carisolv)</td>
<td>21.86 ± 0.35</td>
<td>21.25-22.3</td>
</tr>
<tr>
<td>Subgroup IIC (demineralized dentin + NaOCl)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td>P&lt;0.001, highly significant</td>
<td></td>
</tr>
<tr>
<td><strong>Tukey’s post hoc test</strong></td>
<td>Subgroup IIA&gt;group IIB</td>
<td></td>
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<tr>
<td></td>
<td>P=0.96, Not Significant</td>
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<td></td>
<td>Subgroup IIA&gt; group IIC</td>
<td></td>
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<td></td>
<td>p&lt;0.001, highly significant</td>
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<td></td>
<td>Subgroup IIB&gt; group IIC</td>
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<td></td>
<td>p&lt;0.001, highly significant</td>
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Table 2: The Values Obtained In Terms Of Means For Demineralized Dentin And Demineralized Dentin Exposed To Carisolv And Demineralized Dentin Exposed To 0.25% Sodium Hypochlorite

<table>
<thead>
<tr>
<th>Group III</th>
<th>Mean ± S.D. (μm)</th>
<th>Range (μm)</th>
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<tbody>
<tr>
<td>Subgroup IIIA (denatured dentin)</td>
<td>32.15±0.18</td>
<td>31.89-32.38</td>
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<tr>
<td>Subgroup IIIB (denatured dentin + Carisolv)</td>
<td>20.62±0.34</td>
<td>20.2-21.1</td>
</tr>
<tr>
<td>Subgroup IIIC (denatured dentin + NaOCl)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td>P&lt;0.001, highly significant</td>
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<tr>
<td><strong>Tukey’s post hoc test</strong></td>
<td>Subgroup IIIA&gt;group IIIB</td>
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<td></td>
<td>p&lt;0.001, highly significant</td>
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<td></td>
<td>Subgroup IIIA&gt; group IIIC</td>
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<td>p&lt;0.001, highly significant</td>
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<td></td>
<td>Subgroup IIIB&gt; group IIIC</td>
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<td>p&lt;0.001, highly significant</td>
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Table 3: The Values Obtained In Terms Of Means For Denatured Dentin And Denatured Dentin Exposed To Carisolv And Denatured Dentin Exposed To 0.25% Sodium Hypochlorite