In vitro effectiveness of a chemo-mechanical method for caries removal

T. PERIC, D. MARKOVIC

ABSTRACT. Aim The aim of the study was to assess the efficacy of chemo-mechanical method for caries removal based on histological analysis and scanning electron microscopy of dentine samples. Methods Forty-five freshly extracted human deciduous and permanent teeth with dentinal caries were used in this in vitro study. The teeth were allocated to two different technique groups. Chemo-mechanical caries removal using Carisolv was performed in 30 teeth and in 15 teeth caries was removed using conventional rotary instruments. The caries status of dentine was judged according to clinical criteria (probing and visual inspection). After caries removal had been completed, teeth were sectioned through the prepared cavities and the two halves of each tooth were processed for light microscopy and scanning electron microscopy. Light microscopy was used to examine the specimens for the presence of bacteria in dentine tubules. Scanning electron microscope was used to determine surface characteristics of the cavity floor after caries removal. Results All examined dentine surfaces were clinically caries free. Regarding the presence of bacteria, 53% of chemo-mechanically prepared teeth and 87% of conventionally prepared teeth were bacteria-free (p>0.05). Efficacy of both methods was confirmed using scanning electron microscope, but different dentine topography was recorded after chemo-mechanical and conventional caries removal. Conclusion Based on histological and scanning electron microscope analyses it could be concluded that this chemo-mechanical method is efficient in the removal of carious dentine.

KEYWORDS: Dentine caries, Chemo-mechanical removal, Histology, Scanning electron microscopy.

Introduction

In spite of the development of dental materials and equipment, the problems of rotary instruments for caries removal have remained unsolved. Conventional caries removal has potential adverse effects to the pulp due to heat, pressure and vibration [Sholvelton, 1972]. This approach always results in extensive removal of healthy tooth tissues [Banerjee et al., 2000]. Moreover, drilling often causes pain and requires local anaesthesia, and these procedures are usually perceived as unpleasant especially in children and patients with dental anxiety. Modern restorative dentistry offers numerous options for caries removal that were developed in order to replace rotary instruments. One of these is the chemo-mechanical caries removal method.

Principles of chemo-mechanical caries removal methods are based on studies of Goldman and Kronman [1976]. They initially investigated the possibility to remove carious dentine with a non-specific proteolytic agent, sodium hypochlorite (NaOCl). Due to its high reactivity, NaOCl can dissolve both necrotic and healthy dentine [Hand et al., 1978]. If amino acids are added to NaOCl, this effect is reduced and the action is aimed more specifically at denatured proteins-carious dentine, so that sound and carious dentine become easily separable [Goldman and Kronman, 1976; Schutzbank et al., 1978]. During the treatment several reactions act in concert to disrupt the fibre structure of collagen and have softening effect on the carious tissue, while no changes of the sound dentine are noticed [Cederlund et al., 1999a; Hannig, 1999]. Carisolv is the very last product for chemo-mechanical caries removal. Its active components are three natural amino acids.
(leucine, lysine and glutamic acid) and 0.5% NaOCl. Specific hand instruments are designed to remove softened carious dentine without damaging the healthy dentine.

Several studies report the clinical efficacy of Carisolv method [Ericson et al., 1999; Markovic et al., 2002]. It is claimed to reduce the need for local anaesthesia [Ericson et al., 1999; Markovic et al., 2002] and to decrease the use of rotary instruments [Ericson et al., 1999; Kakaboura et al., 2003; Markovic et al., 2002]. High patients’ acceptance was also pointed out [Ericson et al., 1999; Kakaboura et al., 2003; Markovic et al., 2002]. Various in vitro studies are of utter importance in estimating the effectiveness of new methods for caries removal. Unfortunately, none of the experimental procedures give comprehensive data on the reliability of certain therapeutic procedures. Therefore, obtaining a broad picture necessarily requires a large number of methodologically varied studies. The aim of this in vitro study was to assess the efficacy of a chemo-mechanical caries removal method based on histological analysis of dentine samples and scanning electron microscopic analysis of dentine after the caries removal.

Materials and methods

Forty-five human deciduous (N=33) and permanent teeth (N=12) extracted for exfoliation or orthodontic reasons were selected for this study. Only open, easily accessible, primary coronal caries lesions were used. None of the cavities extended into pulp. All carious lesions were assessed and used by a single operator immediately after the extraction. The teeth were allocated to two different technique groups. Chemo-mechanical caries removal was performed in 30 teeth and in 15 teeth caries was removed using conventional rotary instruments. Independent co-investigator, who was blinded to the method of caries removal, determined the efficiency of the caries removal after cavity preparation.

Carisolv gel for the chemo-mechanical caries removal (MediTeam Dental AB, Gothenburg, Sweden) was prepared according to the manufacturer’s instructions and was applied to the surface of carious lesions. After 30 seconds, the superficial softened layer was removed with specially designed hand instruments. The procedure was repeated until the gel no longer turned cloudy and the surface was hard as judged by clinical criteria (probing and visual inspection).

For teeth treated with drilling, caries was removed with a round steel bur in a slow handpiecife until the cavity was found to be clinically caries free.

After the caries removal had been completed, teeth were sectioned through the prepared cavities in the mesio-distal longitudinal plane using a water-cooled diamond-impregnated circular saw. The two halves of each tooth were processed for light microscopy and scanning electron microscopy.

**Light microscopy.** The specimens were fixed in a solution of 10% buffered formaldehyde for 48 hours, decalcified in 12% EDTA, dehydrated in increasing concentrations of aqueous ethanol, and embedded in paraffin. Serial sections of 5 µm were cut, mounted on glass slides and stained according to the Brown-Brenn [Brown and Brenn, 1931] and Periodic Acid Schiff (PAS) [McManus, 1948] technique. The specimens were analysed with a light microscope (Olympus Bx41TF, Olympus Corporation, Tokyo, Japan) at different magnifications.

Specimens were examined for the presence of bacteria in dentine tubules. Histological findings were scored according to the following criteria: 0 no bacteria detected; 1 bacteria in the cavity floor; 2 bacteria penetrating dentine tubules up to one half of the distance between the cavity floor and the pulp chamber; 3 bacteria in dentine tubules reaching the pulp chamber.

**Scanning electron microscopy.** The specimens were fixed in a solution of 10% buffered formaldehyde for 24 hours, dehydrated in increasing concentrations of ethanol (50%, 60%, 70%, 80%, 96% and absolute ethanol for 60 minutes each), and immersed in hexamethyldisilizane for 15 minutes. The specimens were mounted on aluminium stubs, sputter-coated with gold (Bal-Tec SCD 005 Sputter Coater, Bal-Tec AG, Balzers, Liechtenstein), and then examined using scanning electron microscope (Jeol JSM-6460LV, Jeol Industries Ltd., Tokyo, Japan) at different magnifications to determine surface characteristics of the cavity floor after the caries removal. Fisher’s exact test was used to compare the two caries removal groups and the significance level was p=0.05.

Results

The lesions in the two treatment groups were comparable in terms of dentition, type of tooth, and location (Table 1). According to the independent examiner’s assessment, all examined dentine samples were clinically caries free.

**Light microscopy.** Results of the histological analysis are shown in Table 1. There were no significant differences between the
two groups regarding the presence of bacteria and the depth of their penetration of the dentine tubules (p>0.05, Fisher’s exact test). In the chemo-mechanical group, 53% of prepared teeth were bacteria-free (Fig. 1A). In the remaining teeth, bacteria were observed at various levels of dentine tubules (Fig. 1B, 1C).

Light microscopy also showed irregular, uneven cavity surfaces after the chemo-mechanical caries removal. After conventional caries removal with rotary instruments, 87% of teeth were without bacteria. Walls and floor of cavities were smooth and without irregularities (Fig. 2).

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- 0- no bacteria detected
- 1- bacteria in the cavity floor;
- 2- bacteria penetrating dentine tubules up to one half of the distance between the cavity floor and the pulp chamber
- a- p<0.05 (Fisher’s exact test) between the two caries removal methods

**Fig. 1** - Microscope images showing histological sections after treatment with Carisolv. a. No bacteria colonies can be observed (Brown and Brenn, 20x); b. Bacteria (B) occasionally found in the cavity floor (PAS, 10x); c. Bacteria (B) penetrate deeper parts of dentine tubules (PAS, 20x).

**Fig. 2** - Microscope image showing a histological section after treatment with rotary instruments. The cavity floor is smooth and without irregularities. No bacteria colonies can be observed (PAS, 10x).
Scanning electron microscopy. Scanning electron microscopy confirmed the success of both methods for caries removal, but different morphology of the cavity floor could have been observed. In the chemomechanical group dentine surfaces showed irregular, roughened appearance (Fig. 3A). Several tubule openings were detected all over the cavity (Fig. 3, 4).

Smear layer was minimally formed (Fig. 3B, 4A).

In the group of permanent teeth irregularities were more marked (Fig 4B).

After the caries removal with burs, dentine surfaces showed amorphous structure. Cavity floor and walls were covered with well-formed smear layer (Fig 5B), and dentine tubules were occluded (Fig 5C). Tracks

![Fig. 3 - Dentinal surfaces of primary teeth after treatment with Carisolv. A. SE microphotograph showing irregular cavity surface (1000x); B. SE microphotograph showing patent dentine tubules (PDT) and minimally formed smear-like material (SL) (2000x); C. SE microphotograph showing patent dentine tubules in higher magnification (5000x).](image1)

![Fig. 4 - Dentinal surfaces of permanent teeth after treatment with Carisolv. A. SE microphotograph showing patent dentine tubules (PDT) and minimally formed smear-like material (SL) (1000x); B. SE microphotograph showing extremely irregular cavity surface and patent dentine tubules (5000x).](image2)

![Fig. 5 - Dentinal surfaces after treatment with rotary instruments. A. SE microphotograph showing amorphous dentine structure with tracks from the mechanical preparation (100x); B. SE microphotograph showing well-formed smear layer (1000x); C. SE microphotograph showing occluded dentine tubules (ODT) (2000x).](image3)
from the mechanical preparation could also be seen (Fig 5A). The dentinal surfaces after conventional caries removal did not differ between permanent and deciduous teeth.

Discussion

Chemo-mechanical caries removal is a suitable method in paediatric dentistry, especially in a group of anxious children [Markovic et al., 2002]. Both primary and permanent teeth were combined to evaluate effects of Carisolv method on human primary and permanent dentine and to compare possible differences. Histological analysis did not show differences between deciduous and permanent teeth in both chemo-mechanical and drilling group. However, significant difference between the two technique groups was observed for deciduous teeth (Table 1).

The most widely used criteria for complete caries removal in clinical practice are tactile and visual criteria. However, this method does not always correspond to the amount of carious dentine that should strictly be removed [Meller et al., 2006]. The aim of a modern concept of treatment of dentine caries is to remove only the outer, permanently damaged ‘infected’ layer of carious dentine, but to preserve the demineralised ‘affected’ dentine which can be healed [Kuboki et al., 1977]. In addition, depth of microbial invasion cannot be diagnosed with clinical criteria for complete caries removal [Fusayama et al., 1966]. In the present study 47% of chemo-mechanically prepared teeth and 13% of conventionally prepared teeth showed bacteria at various levels of dentine tubules. It is well known that it is not always possible to remove the bacteria completely from the cavity.

Microorganisms remaining in dentine after the preparation were the subjects of previous microbiological investigations [Björndal et al., 1997; Kidd et al., 1993], which showed that a low number of residual bacteria (below 102 CFU) could often be found in clinically caries free dentine. However, authors state that this number of microorganisms could be considered as clinically acceptable because those bacteria will not invade the dental pulp. Fisher [1966] showed that lactobacilli, staphylococci and streptococci could persist under the restorations for a considerable time. However, it was pointed out that conditions are unfavourable for bacterial growth and metabolism when they are separated from the oral environment. Bacteria under the proper restorations have altered morphological characteristics and tend to die out [Shovelton, 1972].

In the present study histological analysis showed slightly higher occurrence of bacteria after the chemo-mechanical caries removal compared to the rotary instruments. This finding could be explained by less extensive preparation compared to the one with rotary instruments. Chemo-mechanical method preserves the sound dentine where remaining bacteria can be found. On the other hand, Yazici et al. [2003] explain the presence of bacteria by the absence of smear layer in the chemo-mechanically treated cavities, which enables direct pushing of bacteria into the dentine tubules with hand instruments.

The present study used the traditional method for preparation of the specimens which includes decalcification of hard tooth tissues. During the decalcifying procedure dentinoenamel junction is lost [Cederlund et al., 1999b], so it was not possible to analyse this structure. In the histological study of Cederlund et al. [1999b], residual caries was not observed in dentine after the chemo-mechanical caries removal, while it was a frequent finding at the dentinoenamel junction (6 of 10 cavities). Fluckiger et al. [2005] showed that Carisolv method had a tendency to leave carious dentine close to the dentinoenamel junction. Yazici et al. [2003] reported more frequent presence of bacteria after the Carisolv treatment (9 out of 14) compared to the conventional rotary instruments (1 out of 14), especially at the dentinoenamel junction. This is why it is very important to assure the direct access to the dentinoenamel junction. Carisolv gel cannot act chemically to the undermined enamel, and in that case carious lesion will not be exposed to the gel enough.

Light microscopy showed irregular, uneven cavity surfaces after the chemo-mechanical caries removal. This finding was confirmed by scanning electron microscopy.

Results of this paper agree with those of earlier SEM studies of permanent dentine characteristics after the chemo-mechanical removal [Banerjee et al., 2000; Sakoolnamarka et al., 2002; Splieth et al., 2001]. Those investigations showed irregular, porous surfaces with opened dentine tubules. The smear layer was minimally formed [Banerjee et al., 2000; Splieth et al., 2001] or could have not been observed [Sakoolnamarka et al., 2002]. Hosoya et al. [2001] investigated the influence of Carisolv treatment on sound human primary and young permanent dentine and found Carisolv to be more effective in removal of the smear layer in primary dentine than in permanent dentine. This could be accounted for by the differences in mineralization and dentinal micromorphology (i.e.
concentration and diameter of dentine tubules) between primary and permanent teeth [Hosoya et al., 2001]. However, the present study does not confirm differences in removal of smear layer between deciduous and permanent dentine. The absence of a smear is a result of the specific preparation technique without thermal or mechanical effects, the high pH of the gel [Banerjee et al., 2000], and the proteolytic action of NaOCl [Hannig, 1999]. On the other hand, Cederlund et al. [1999a, 1999b] reported smooth, smear-like dentine surfaces with occluded tubules after the treatment with Carisolv. These findings cannot be explained, since the protocols of investigations of Cederlund et al. were the same or similar to the other studies of dentine surfaces after the chemo-mechanical caries removal [Banerjee et al., 2000; Sakoolnamarka et al., 2002; Spleith et al., 2001].

In the operative treatment of carious lesions in dentine, the morphology and nature of the prepared dentine surface influences bonding of adhesive restorative materials [Eick et al., 1997]. After mechanical caries removal with rotary instruments, an amorphous layer is formed on the surface of the dentine [Banerjee et al., 2000; Sakoolnamarka et al., 2002; Spleith et al., 2001]. The presence of smear layer can affect the adhesion of modern dental materials [Eick et al., 1970]. Thus it has to be removed or modified prior to the placement of the restoration. The fact that the dentine surfaces are irregular, without an extensive smear layer and with open tubules induced a thought that these characteristics could improve bonding between restoration and tooth [Banerjee et al., 2000]. However, the microtensile bond strengths of different adhesive systems did not differ between the chemo-mechanical and conventional treatment with burs [Haak et al., 2000]. Less data are available about different caries excavation methods on adhesion to caries-affected human dentine after four different caries excavation. J Dent 2003; 48:110-4.


