BACTERIOLOGIC EVALUATION OF RESIDUAL DENTIN AFTER CHEMO-MECHANICAL CARIES REMOVAL IN PRIMARY TEETH

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REVIEW OF LITERATURE

Caries can be defined as localized demineralization and tissue breakdown of the hard tooth structure caused by organic acids produced by the bacteria. Acid producing bacteria such as Streptococcus mutans and Lactobacilll are considered the main causative organisms. Bacterial enzymes may play an important role in dentin caries (Thylstrup and Fejerskov, 1994).

Although caries has affected humans since prehistoric times, the prevalence of this disease has greatly increased in modern times on a worldwide basis, an increase strongly associated with dietary change (Sturdevant 2002).

According to the World Health Organization, 2006 dental caries remains a major public health problem in most high income countries, affecting 60-90% of schoolchildren and the majority of adults. It is also the most prevalent oral disease in several Asian and Latin American countries.

When caries occurs, acids 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 demineralized, the collagen and other matrix components are then susceptible to enzymatic degradation. With respect to collagen degradation, two zones can usually be distinguished within a lesion. There is an inner layer, known as affected dentin, and there is an outer layer, known as infected dentin (Ogushi and Fusayama, 1975).
Table (1): Differences between infected and affected dentin.

*(Ogushi and Fusayama, 1975)*.

<table>
<thead>
<tr>
<th>Infected dentin</th>
<th>Affected dentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial layer in the carious lesion.</td>
<td>Below the superficial infected layer.</td>
</tr>
<tr>
<td>Wet aspect and soft consistency.</td>
<td>Hard and leathery consistency.</td>
</tr>
<tr>
<td>Highly infected by bacterial penetration.</td>
<td>No bacterial penetration, only toxin penetration.</td>
</tr>
<tr>
<td>Irreversible degradation of collagen fibers.</td>
<td>Partially demineralized, collagen fiber still intact.</td>
</tr>
<tr>
<td>No possibility of re-mineralization.</td>
<td>Can be re-mineralized.</td>
</tr>
<tr>
<td>Stained by caries detector dye.</td>
<td>Cannot be stained by caries detector dye.</td>
</tr>
<tr>
<td>Must be removed.</td>
<td>Can be preserved.</td>
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</tbody>
</table>

Dentin is the best protective barrier for the pulp so it’s better to allow a layer of discolored dentin to remain for the protection of pulp rather than run the risk of sacrificing the tooth. The inner carious dentin does not permits bacterial penetration because its dentinal tubules are filled with odontoblastic processes and should be preserved during clinical treatment *(Yamada et al., 1983 and Fusayama, 1997)*.

An efficient process of caries removal should identify the mineralized portion as well as the demineralized one, and remove only the latter. For these we require reagent, which must be able to cause further degradation of this partially degraded collagen, by cleavage of the polypeptide chains in the triple helix and/or hydrolyzing the cross linkages *(Ericson, 1998)*.

Collagen is an unusual protein which contains 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 the tropocollagen units form cross links and give the collagen fibrils stability (Beeley et al., 2000).

The best way to ensure a maximum life for the natural tooth is to respect the sound tissue and protect it from damage by using minimally invasive techniques in restorative dentistry (Banerjee et al., 2000b).

Minimally invasive dentistry reaches the treatment objective using the least invasive surgical approach, with the removal of the minimal amount of healthy tissues. It includes the following different techniques: Air abrasion, Sono abrasion, Laser and Chemomechanical caries removal (CMCR) (Ganesh and Parikh, 2011).

**Techniques of caries removal:**

Before 1870, dentists had no rotary instruments to be used for caries removal. The procedure of overhanging enamel cleavage was taken by enamel cutters while the caries was scooped out with excavators (Yip and Samaranayake, 1998).

In 1871, James Morrison’s treadle instrument was developed from the mechanism of Isaac Singer’s sewing machine, based on his foot engine concept. Modern high speed drills are the latest development of this more than a century old techniques. Effective rotary instruments for removal of enamel (10,000 rpm) have not been available till 1947 (Beeley et al., 2000).
**Hand Instruments:**

Hand cutting instruments are manufactured from two main materials: carbon steel and stainless steel. In addition, some instruments are made with carbide inserts to provide more durable cutting edges. Carbon steel is harder than stainless steel, but when unprotected, it will corrode. Stainless steel remains bright under most conditions but loses a keen edge during use much more quickly than does carbon steel. Carbide, although hard and wear resistant, is brittle and cannot be used in all designs (*Sturdavent, 2002*).

Hand cutting instruments are used to cut hard or soft dental tissues. Excavators are used for removal of caries and refinement of the internal parts of the preparation. Chisels are used primarily in cutting enamels. Excavators are subdivided into hatchets, hoes, angle formers and spoons, the cutting edges of the spoons could be circular and known as discoid excavators, or claw like and known as cleoid excavators. Chisels could be grouped into: a) straight, curved and bin-angled chisels. b) Enamel hatchets. c) Gingival trimmers (*Sturdavent, 2002*).

Dentin excavation was accomplished in circular scratching movements from the dentinoenamel junction to the cavity floor, using spoon excavators according to the lesion size. Excavation was completed when dentin at the cavity floor was resistant to probing, following the clinical criteria of hard texture (*Cláudia et al.,2006*).
**Rotary instruments:**

The rotary instruments were classified according to the speed range into: low speed (below 12,000rpm), medium speed (12,000 to 200,000) and high speed (above 200,000). The low speed range is used for occasional caries excavation, cleaning teeth and finishing and polishing procedures. The high speed is used for tooth preparation and removing old restorations. At high speed, the cutting of the tooth structure is more efficient, faster with less pressure, less vibration, less heat generation, more patient acceptance, less patient apprehension and more operator control. The main disadvantage is the rapid removal of the sound uninfected dentin. As tactile sensation decreases with increasing speed, sound uninfected dentin may be removed, resulting in an excessive loss of sound tooth structure (*Heyeraas et al., 2001 and Sturdavent, 2002*).

With the improvements in the modern mechanical cutting devices, some problems were encountered. As drilling usually necessitates the application of local anesthesia which is another aspect of dental treatment that render patients particularly anxious. Many factors were also found to contribute to the iatrogenic pathological changes induced in dentin and pulp. These include speed, desiccation, heat, pressure, cutting time, depth of the cavity and area of prepared dentin (*Yip and Samaranyake, 1998 and Mubarak, 2003*).

**Air abrasion:**

Air abrasion was developed in 1945 by R.B.Black. He investigated an alternative, non-rotary, mechanical method for caries removal. It involved bombarding the tooth surface with high velocity aluminum oxide particles on a stream of air (*Yip and Samaranyake, 1998 and Banerjee et al., 2000c*).
The KCPS (Kinetic Cavity Preparation System), which uses a high velocity stream of alumina particles (alpha alumina) was promising clinically after studying the surface topography of enamel and dentin following cavity preparation with the air abrasion technique. Bakry in 2000 found that air-abraded enamel and dentin, of both permanent and primary teeth, were irregularly rough and that a smear layer was created on the air-abraded dentin.

This technique shows a dramatic decrease in the heat, vibration and the mechanical stimulation resulting in nearly pain free procedures, soundless and supposed to improve the tooth surface for bonding. Some disadvantages were encountered as: loss of tactile sensation, inadequate caries removal, ineffectiveness in removing the filling materials and pollution of the operating room by dust. It cannot be used for onlay or crown preparations, and the neighboring teeth should be covered with a rubber dam which makes the procedure more complicated (Mubarak 2003).

Recently it was proved that air abrasion system removes sound dentin more efficiently than carious dentin (Paolinelis et al., 2006).

**Sono abrasion:**

The main advantages of this technique are the less heat, vibration, noise and high patient acceptance. But the limited instrument tips, slow action, poor visibility and the maintenance problems were the main drawbacks of this system (Mubarak, 2003).

Recently, the technology for diamond growth by chemical vapor deposition (CVD) has been used for the fabrication of new dental burs.
These burs offer better cooling, present less noise, greater durability and better access and visibility (*Lima et al.*, 2006 and *Predebon et al.*, 2006).

However the performance of the high speed handpiece was better than that of the oscillatory system (*Vieira et al.*, 2007).

**Laser:**

Laser is an acronym for “light amplification by stimulated emission of radiation”. The first ruby laser was developed in 1960, since that time many modifications were carried out to use laser in dental applications. Laser devices produce beam of coherent and intense light, this light is transmitted into the application site via a flexible fiber-optic light pipe, where it is normally focused by a lens to a focal area near the tip (*Sturdevant*, 2002).

Several lasers are of practical importance to dentistry such as: Nd-YAG, Er-YAG and CO₂. Argon, Helium-neon, Ho:YAG and Excimer lasers are being evaluated as well (*Sturdevant*, 2002).

Advantages compared to regular drilling involve less pain, noise, pressure, vibrations and sterile cut surface. The main disadvantages are high price, cannot be used on intraoral metals and it must be used with safety precautions, such as: closing off the room when the laser is being used, eye protection for the dentist, patient and assistants as well as avoidance of using any metal or reflecting objects in the field of laser application (*Sturdavent, 2002*).
In terms of carious dentin removal, CO$_2$ laser was reported to act better on enamel and dentin irrespective of their color, Nd:YAG laser allowed finer control, while Er:YAG laser was found to be effective in hard tissue ablation with less energy loss in the surrounding tissues. The ultraviolet emission of Excimer laser was found to be more selective in caries removal, also, the use of dye enhanced laser ablation help to remove caries selectively. In the presence of a suitable laser sensitizier, the low power laser has the ability to destroy the Streptococcus mutans, fuse the Hydroxyapatite to enamel and dentin and form a biological seal for pits and fissures (Parkins 2000 and Mubarak 2003).

El-Dokky in 2006, studied the effect of CO$_2$ laser and conventional method on early carious lesion in primary teeth, sixty extracted teeth were included in this study& divided into two groups. Surface assessment of prepared cavities lased and unlased done by scanning electron microscope. Microhardness measurements of lased and unlased surfaces was taken by microhardness tester. it was found that of CO$_2$ laser had ability to increase enamel and dentin density values and increase microhardness of lased dentin.

Enzymes:

Goldberg and Kiel, in 1989, reported that the bacterial collagenase enzyme gained from Achromobacter species is useful in removing soft carious dentin when the enzymes are left for about 48-92 hours, while the sound dentin underneath could be left without affection. No residual bacteria were detected after using this enzyme. Another study reported that pronase enzyme, which is a non specific proteolytic enzyme extracted from Streptomyces griseus, showed the same results. These techniques need further in vitro investigation (Banerjee et al., 2000c).
Chemomechanical caries removal:
Chemomechanical caries removal method offers an effective alternative to mechanical caries removal because it brings atraumatic characteristics together with a bactericidal and bacteriostatic action (Beeley et al., 2000).

Singh et al., in 2000 proved that children who were submitted to local anesthesia during dental treatment demonstrated more fear than those who were not submitted to local anesthesia.

Chemomechanical caries removal does not require local anesthesia during the procedure since the carious tissue is softened by the gel and its removal by gentle hand instruments does not promote any stimulus or pressure that would lead to discomfort and/or pain (Pereira et al., 2004).

Wang and Wang in 2007 evaluated the effects of three caries removal methods on children's dental fear by physiological measure on 90 children with caries lesions into dentin in primary molars were divided into three groups randomly: Chemo-mechanical carious removal group, atraumatic restorative treatment (ART) groups and traditional rotary instrument group. The baseline of blood pressure, pulse was recorded before the treatment. Then the blood pressure and the pulse of each subjects were measured at the end of the treatment respectively. Compared to traditional caries removal method, chemo-mechanical technique and ART may decrease children's dental fear effectively.
Goldman and Kronman, In 1976 reported on possibility of removing carious material chemically using N-monochloroglycine (NMG, GK101). Subsequently, after modification, the Caridex system containing N-monochloro-D, L-2 aminobutyrate (NMAB, GK101E), was introduced. Eventually shortcomings with Caridex became apparent because of its efficacy and speed of caries removal needed improvement. It was expensive and difficult in application with short shelf life.

Dan Ericson and Rolf Bornstein worked in the development of a new system for chemo-mechanical caries removal called Carisolv. It has been approved for clinical dental use by the Swedish counterpart to the FDA and is currently available in the market (Beeley et al., 1999).

Carisolv comprising two parts: Carisolv gel and Carisolv hand instruments. Carisolv gel consists of a red gel and a transparent fluid. Equal parts of the two are mixed to form the active gel substance. The red gel primarily contains three different amino acids (glutamic acid, leucine and lysine) and sodium hydroxide. The transparent fluid contains the reactive hypochlorite component (NaOCl). Special Carisolv hand instruments are used to remove the carious tissue layer by layer (Carisolv, 2007).

The gel is applied to the carious lesion with one of the hand instruments and after 30 seconds, carious dentine can be gently removed. The procedure is repeated until no more carious dentine remains until the gel removed from the tooth is clear. The time required for the procedure is about 9 to 12 min (Carisolv, 2007).
Banerjee et al., in 2000a analyzed the different surface characteristics of the dentin cavity floor created after preparation using five different methods of excavation: hand excavation, slow-speed bur, sono-abrasion, air-abrasion and Carisolv gel. Fifty cavities analyzed using scanning electron microscopy (SEM) to ascertain the surface characteristics of the dentin at the cavity floor. Carisolv gel was the only method examined that consistently removed the smear layer during excavation to leave exposed dentine tubules at the end of cavity preparation.

Hahn et al., in 2001 investigated the dentin surface after caries removal using Carisolv gel in comparison to round rotary bur. Twenty six permanent human carious teeth were used. Representative samples from each group were scanned using SEM. The dentin surfaces had a rough and mat appearance when treated using Carisolv. On the other hand, Bur usage caused smear layer formation with smoother dentin surfaces.

Maragakis et al., in 2001b evaluated the clinical efficiency and patient acceptance of the Carisolv in deciduous teeth. It was found that Cariosolv was effective in removing infected carious tissue but it did not prove to be a practical alternative to drilling. This was mainly because of its high cost, need of special instruments and more time consuming. Also children dislike its chlorine taste and odor.

Azrak et al., 2004 and Naga, 2006 proved that Carisolv reduced the cariogenic flora to the same extent as conventional hand excavation.
New chemomechanical caries removal agent (Papacárie):

Though carisolv is the most successful agent, it has its own share of disadvantage which includes extensive training and customized instrument which increases the cost of the solution and there was a restriction in its application. To overcome these disadvantages of carisolv system, a new agent was developed. In Brazil 2003, Formula eacao by Sao Paulo, first time introduced papain gel as papacarie for chemomechanical caries removal agent. Its main components are papain, chloramine and toluidine blue (Bussadori et al., 2005).

Composition and biological properties of Papacárie:

Papain, chloramine, toludine blue, salts, preservatives, thickener, stabilizers, deionized water (Papacárie, 2010).

Papain is the main ingredient of Papacárie, a gel used for chemomechanical dental caries removal. (Papacárie, 2010).

Papin consists of 212 amino acids stabilised by 3 disulfide bridges. Its 3D structure consists of 2 distinct structural domains with a cleft between them. This cleft contains the active site, which contains a catalytic triad that has been likened to that of chymotrypsin. Its catalytic triad is made up of 3 amino acids - cysteine-25 (from which it gets its classification), histidine-159, and asparagine-1 (Camphuis et al., 1984).

Papain is a cysteine protease, hydrolase enzyme present in papaya (Carica papaya) and mountain papaya (Vasconcellea cundinamarcensis) (Lohiya et al., 2002).
The papaya (from Carib via Spanish) is the fruit of the plant *Carica papaya*, in the genus *Carica*. It is native to the tropics of the Americas, and was cultivated in Mexico several centuries before the emergence of the Mesoamerican classic cultures. It is sometimes called a "big melon" or a "paw paw" but the North American pawpaw is a different species, in the genus *Asimina* (*Lohiya et al.,* 2002).

Carica papaya has significant antibacterial activity against both gram-positive and gram-negative bacteria ( *S. aureus*, *E. coli*, *B. cereus*, *P. aeruginosa* and *S. flexeneri*). Also carica papaya has bactericidal and bacteriostatic properties (*Emeruwa, 1982* and *Dawkins et al., 2003*).

Papain, a proteolytic enzyme, reacts with the partially decayed collagen of the necrosed tissue of the carious lesion only which lack of plasmatic antiprotease called alfa-1-antitrypsin. The alfa-1-antitrypsin is only present in sound tissue. The alfa-1-antitrypsin is an antiprotease which prevents the action of the proteolytic enzymes and inhibits protein digestion. Papain facilitate the cleaning of both necrotic tissues and secretions. As a result, it decreases the time required for tissue recovery and does not damage the sound tissues around the lesion (*Flindt, 1979* and *Papacário, 2010*).

Chloramine (monochloramine) is a chemical compound with the formula NH₂Cl. It is usually used as a dilute solution where it is used as a disinfectant. The term chloramine also refers to family of organic compounds with the formulas R₂NCl and RNC₁₂ (R is an organic group) (*White, 1992*).

Chloramines are compounds that contain chlorine and ammonia. They have bactericidal and disinfectant properties that are used to irrigate
root canals. It is also an additional chemical softener of the carious dentin. The degraded or partially degraded portion of the carious dentin collagen is chlorinated. This chlorination affects the secondary and or quaternary structure of the collagen by breaking the hydrogen bonds thus facilitating it’s removal (Maragakis et al., 2001a).

Chloramine promotes the collagen chlorination of the decayed dentin and acts only on the necrosed portion of the tissues; the healthy tissue is preserved (Papacárie, 2010).

Toluidine blue was added as a coloring agent. It is also a powerful antimicrobial agent that fixes itself to the bacterial wall. As most of oral bacteria do not absorb the visible light, the addition of toluidine blue potentiates the antimicrobial action of the gel when the technique is associated with the use of a visible light (Silva et al., 2004).

Papacárie has a synergic action due to effect of papain and chloramine on the softening of the necrosed portion of the decayed tissue, thus facilitating its removal and preserving the healthy dentin over which it has absolutely no effect, whether it be the dentin or the dentinal pulp. No special instrument is necessary to apply the product: the softened dentin is scraped away following the regular curettage procedure, using an old, blunt curette, or the opposite edge of a curette, so that only the softened dentin and the gel are removed and the healthy dentin is preserved uncut by the instrument (Papacárie, 2010).

**Cytotoxicity of Papacárie:**

*Miyagi et al., in 2008* analyzed in vitro study the cytotoxicity of Papacárie using human pulp fibrblasts. The cell viability percentages
were obtained 50 seconds and 24 hours after the cell contact with Papacárie. The result compared with the control groups. They reported that Papacárie gel is a biocompatible product.

**Martins et al., in 2009** carried out a study to compare the cytotoxic effects of Papacárie and Carisolv, to a nontreatment control on cultured fibroblasts in vitro and to study the biocompatibility of the tested substances implanted in subcutaneous tissue of rats in vivo. There were no differences in cell survival between the control and experimental groups. The histological analysis revealed a moderate inflammatory response in both experimental groups. The two tested substances exhibited acceptable biocompatibilities and demonstrated similar responses in the in vitro cytotoxicity and in vivo implantation assay.

**Clinical and laboratory studies on Papacárie:**

**Ammari and Moliterno, in 2005** compared between two products Carisolv and Papacárie for the chemo-mechanical caries removal. It was stated that the two products have the same indications for use and the same mechanism of action. They produced a similar selective proteolytic effect on infected dentin. Regarding their application technique, the Carisolv requires special kit of curettes while with Papacárie, any curette could be used.

**Motta et al., in 2007** carried out a case study by using papacarie with restorative composite resin Filtek on anterior deciduous teeth. they concluded that Papacárie is an appropriate procedure for aesthetic solution in pediatric dentistry.

**Araújo et al., in 2007** evaluated the marginal sealing of adhesive restorations after removal of carious tissue with papain gel and after
conventional method on seventeen extracted human premolars with carious lesion involving occlusal & proximal surface. It was reported that the chemo mechanical method of removal of carious tissue with papain gel influenced the degree of leakage and compromising the marginal sealing of the adhesive restorative system employed.

**Basting et al., in 2007** evaluated the shear bond strength of an adhesive restorative system on sound and demineralized dentin after the use of a Papacârie gel. Forty human dentin slabs were randomly distributed into four groups: sound dentin slabs that received an Papacârie gel, sound dentin slabs that did not receive a Papacârie gel, demineralized slabs that received the agent and demineralized slabs that did not receive the agent. The slabs were restored with resin composite. There were no differences in the shear bond strength means among the groups. The use of a Papacârie gel to remove dental caries did not interfere in the bond strength of restorative materials to dentin.

**Corrêa et al., in 2007b** evaluated the hardness of the remaining dentinal surface after carious tooth tissue removal with a low speed conventional bur and two chemo-mechanical methods, (Papacârie and Carisolv). It was concluded that the hardness of the remaining dentin after carious tissue removal was lower than that obtained on healthy dentin, without significance between the different means of carious tissue removal.

**Carrillo et al., in 2008** evaluated complete caries removal time and patient acceptance of the chemo-mechanical caries removal agent Papacârie in disabled patients. Patients were divided into two groups: children with or without visual or hearing impairments, motor disability and inability to respond to simple orders; and children, without visual