

Antimicrobial Efficacy of Newly Prepared Experimental Chemomechanical Caries Removal Agent

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ABSTRACT

Chemo Mechanical Caries Removal (CMCR) is an effective alternative method of caries removal as they cause less pain, discomfort and less demand of anesthesia when compared with conventional caries removal. The aim of this study was to estimate the antimicrobial efficacy of the newly prepared papa in-base caries removing agent.

Materials and Methods: Thirty five human permanent molars with class I cavity were collected according to specific clinical and radiographic criteria adopted by the researcher. These teeth were subjected to CMCR, using an experimental chemomechanical caries removing agent (ECMCRA) in comparison with Carisolv[®]. The dentine samples were analysed for total bacterial count (TBC), Oral Streptococcus spp. (OSC) and Oral Lactobacillus spp. count (OLC) before and after caries removal with ECMCRA and Carisolv[®].

Results: Student T-Test indicated significant reduction in TBC after caries removal with either agents, 77.1% of samples presented no longer of OSC after caries removal with ECMCRA and 80% after Carisolv[®]. OLC shows 60% elimination after caries removal with ECMCRA and 62.8% after caries removal with Carisolv[®]. The effect of ECMCRA and Carisolv[®] comparable and no significant differences between them.

Conclusion: Antibacterial effect of ECMCRA proved to be efficient and comparable to Carisolv[®] from bacteriological point of view.

Keywords: Chemo Mechanical Caries Removal, colony forming unit, caries removing agent, total bacterial count, Oral Streptococcus spp. and Oral Lactobacillus spp. Count.

INTRODUCTION

Dental caries is the most common pathological change of dentine characterized by cavitation of the enamel and penetration of microorganisms into the dentine is caused by disequilibrium between demineralization and remineralization of hard tissues induced by the proliferation of cariogenic bacteria and consequent increase in acid production, causing the saliva pH to drop to a critical level ⁽¹⁾.

Takao Fusayama in 1979 distinguished two layers in caries lesions. The first layer “outer carious dentine” is highly infected, acidic, demineralized, not sensitive to contact, can be removed without anesthesia and fail to remineralize in a

natural way and the second layer “inner carious dentine” is partially demineralized and slightly infected, but the collagen fibrils retained their natural structure around intact dentinal tubules ⁽²⁾.

Dental caries treatment is very often associated with pain and fear, it is estimated that 80% of all dental patients are apprehensive. Often this apprehension is due to the vibration, noise, pain stimulus experienced with the use of drill during caries removal ^(3,4,5).

The traditional caries removal method involves local anaesthesia followed by the use of burs in low and /or high speed handpieces ⁽⁶⁾.

This method may be traumatic to the pulp due to pressure, thermal damage and vibration ^(7,8). The incidence of pulpal alterations due to the pressure or heat generated by the burs have been reported ^(9,6).

Moreover, its use equally removes infected and altered affected dentine due to the cutting efficiency of the bur, resulting in excessive loss of healthy tooth structure ^(10,911).

The chemomechanical caries removal methods (CMCR) appeared as an alternative, overcoming some of the inconvenient aspects of drilling, such as pain and discomfort, eliminating or diminishing the need for local anaesthesia, and eliminating the noise during carious tissue removal ^(6,11).

MATERIALS AND METHODS

The newly prepared ECMCRA composed of papain enzyme as main active ingredient, chloramine, potassium oxalate, toluidine blue, sodium methyl paraben and sterile water. Thirty five human permanent molars with class I cavity were collected from patients age (18-30 years). Reasons for extraction were periodontal disease, orthodontic problems and wisdom molars or for prosthetic reasons.

A brief history was recorded and the subjects were not taken any antibiotics or antiseptic mouth wash for the last month before extraction and had to have permanent molar with a broad opened occlusal cavitated lesion, showing brown and softened dentine and so the carious dentine was accessible without any drilling. Radiographically, the carious lesion had to be clearly visible as a radiolucency extending into, but confined to the outer dentine of the occlusal surface with no periapical lesion. Each tooth was handled aseptically as soon as possible after extraction and split in two halves along its long axis through the middle of the carious lesion using a sterile water-cooled diamond disk mounted in slow speed handpiece.

The cavity irrigation with sterile saline (0.9% normal saline, Pro-FleksBolu. Turkey) solution to remove any superficial debris and food ruminants, then drying with pieces of sterile cotton, the first sample of carious dentine was collected with the aid of a No. 17 dentinal curette, the carious dentine portion of the bottom wall of the cavity was removed and placed in a glass flask containing 1ml of transport medium thioglycolate broth (Hi Media Laboratories Pvt. Ltd. India). Figure 1 illustrate the outline of the procedure.

The carious lesion of each half were removed with either ECMCRA or Carisolv[®] (MediTeam Dental Sweden) and the second samples of remaining dentine were obtained after caries removal as in the first sample. The protocol of caries removal done by thoroughly soaked the lesion by the gel, After waiting for the gel to act, when the gel turned cloudy and the softened dentine was excavated with a select Carisolv[®] instrument to match the size of the cavity. Scrape off the superficial softened carious dentine. Fresh gel was then applied and the cavity floor was repeatedly scraped until the gel became clear. Caries excavation of the was judged complete by tactile criterion, by checking with a sharp dental explorer, until a sharp scratching sound was heard.

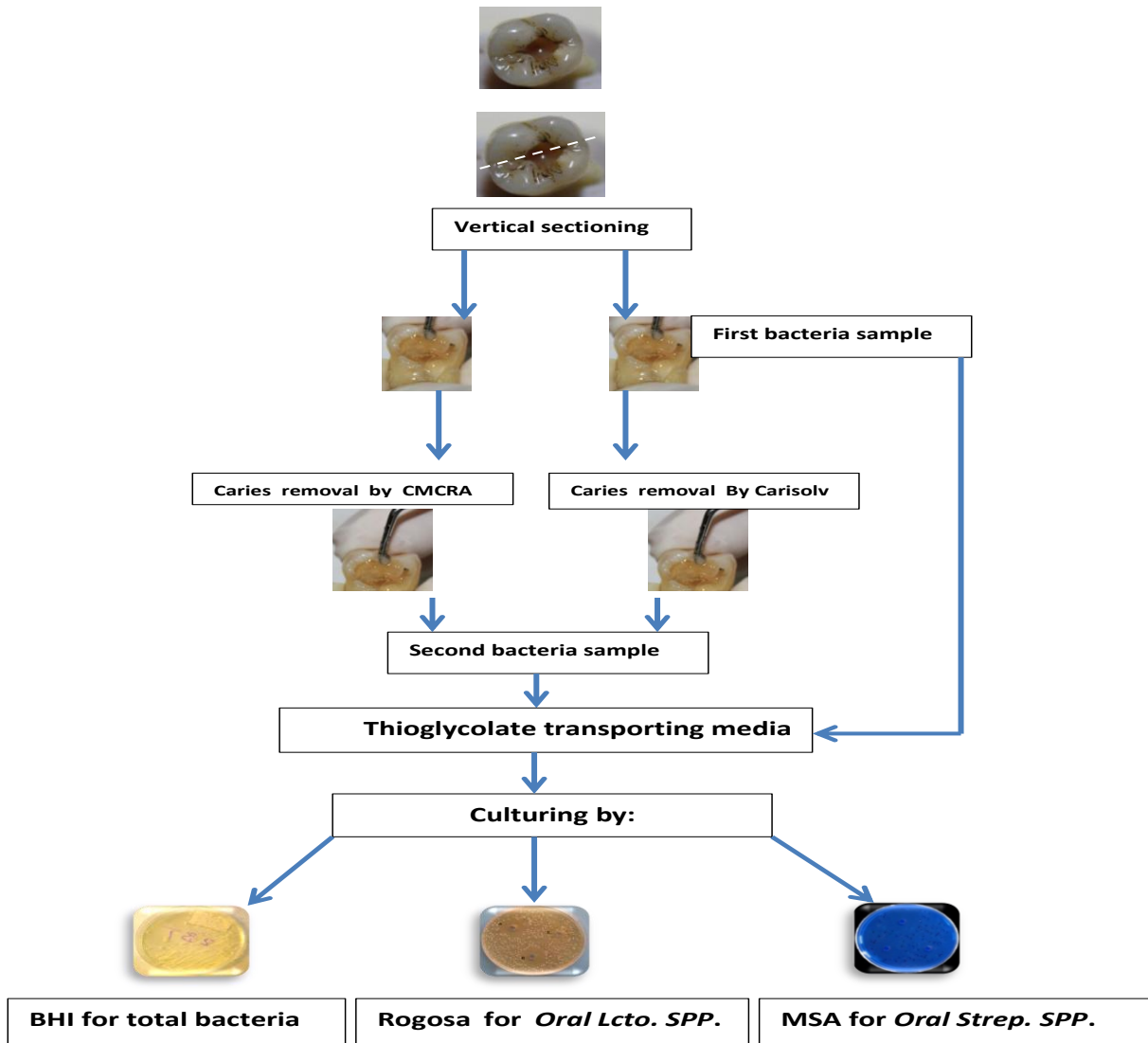


Figure 1: A schematic drawing illustrating the experimental procedure to determine the total bacteria count, OralStrep. Spp. and Oral Lacto. Spp. count.

In order to test the accuracy of the sampling, the weight of the dentine samples was determined. The procedure was as follows: a sterile glass flask containing 1ml of transport medium thioglycolate broth was first weighed on digital sensitive balance (PGW 153i, Germany) accuracy 0.001mg. With the aid of a No.17 dentinal curette, the carious dentine piece was removed and add to the flask until 1mg±0.001 was obtained, by subtracting of the final weight with carious dentine from the early weight of flask and broth. The number of bacteria obtained for a given amount of dentine was used to estimate the number of bacteria present in 1mg dentine (colony-forming units, CFU/mg)⁽¹²⁾.

Immediately after removal, the dentine samples were transported to the Laboratory of Microbiology/ University of Mosul/ College of Dentistry/ Department of Basic Sciences were processed within 2hrs after collection.

Each sample was vortexes for about 20s in order to dislodge the bacteria from the dentine. The dentine samples were submitted to quantitative culture for total bacterial count (TBC), Oral Streptococcus spp. (OSC) and Oral Lactobacillus spp. count (OLC)⁽¹³⁾.

Decimal dilutions were then prepared in sterile saline (0.9% NaCl). Next, 0.1ml Aliquots of each dilution were spread, in duplicate, on the following solid media: brain-heart infusion agar (BHI)(Hi Media Laboratories Pvt. Ltd. India) was used to determine the TBC (Lula et al.,2009). Mitis-Salivarius agar (MSA) supplemented with 0.2 IU/mL bacitracin and 15% sucrose was used for the OSC⁽¹⁴⁾ and Rogosa medium was used for (Hi Media Laboratories Pvt. Ltd. India) OLC⁽¹⁵⁾.

The Rogosa and brain-heart agar plates were incubated under anaerobic conditions for 48hrs^(16,17), whereas the MSA plates were incubated in an atmosphere of 5% CO₂ with gas pack for 48hrs^(14,18).

Up to 5 colonies were selected from each culture medium and from the selected dilutions, and submitted to gram stain to confirm bacterial identification under microscopic examination.

After incubation, the number of colony forming units/mg dentine (CFU/mg) was determined for the first and second samples by using the number of colonies in a given dilution, the inverse factor of the dilution selected for the count and the correction factor of the inoculated volume (100). The number of CFU was determined and calculated on plates containing 10–300 colonies⁽¹²⁾.

Statistical analysis was conducted using ANOVA. Tests of differences between the treatments were analysed by Student T-Test, and a value of P < 0.05 was considered statistically significant.

RESULTS

The TBC in carious dentine samples before and treatment ranged between 5.1X10⁴ - 8.8X10⁷ CFU/mg. After treatment with Carisolv, the dentine presented counts that ranged from 2.1X10² - 6.5X10⁴ CFU/mg, while after treatment with ECMCRA the dentine count ranged from 2 X10² - 5.6 X10⁴ CFU/mg. Comparison of the TBC for carious lesions before and after treatment with Carisolv and ECMCRA by the Student T-Test shows significant reduction in TBC and both gels are comparable, since there were no significant differences between them (p=0.1) Fig.(2).

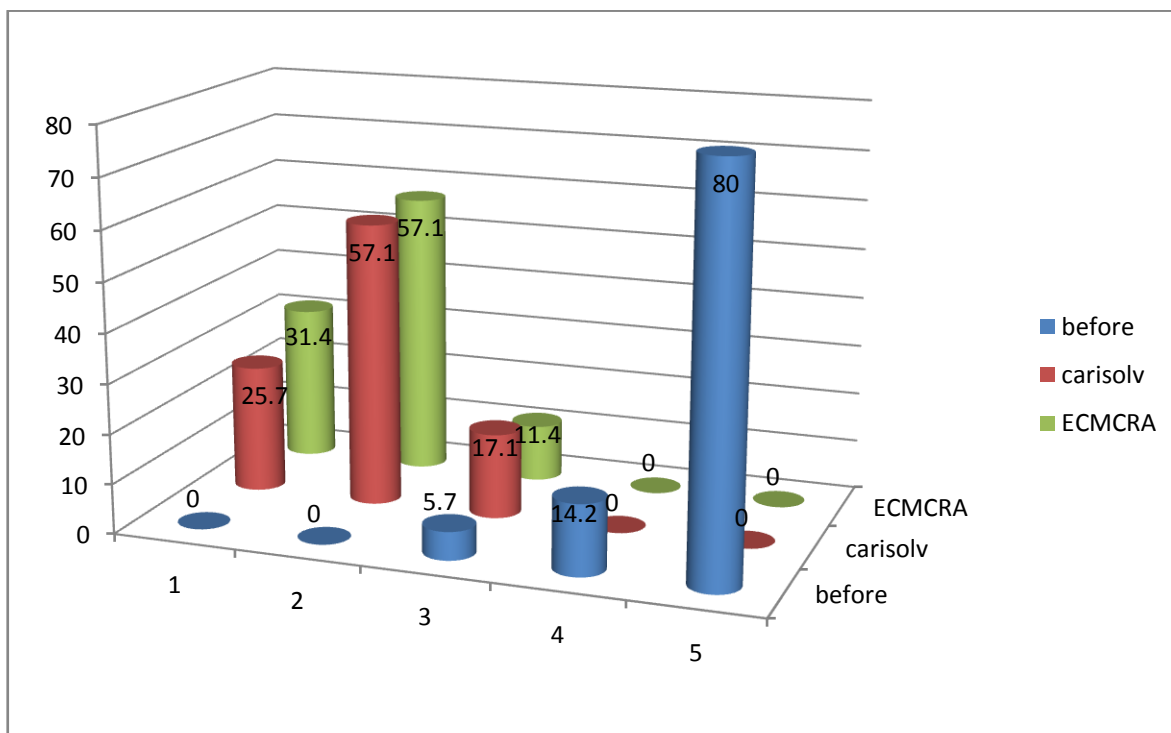


Figure 2: Percentage of total bacterial count for carious lesions before treatment and after treatment with Carisolv and ECMCRA in class 1≤10² ; 2:10¹-10³ ; 3:1001-10⁴ ; 4:10 001-10⁵ ; 5:>10⁶ .

For the (OSC) before treatment, presented carious dentine counts between 5.4×10⁷–2.2×10³CFU/mg. After treatment with Carisolv, the dentine presented OSC that ranged from 0–2.3×10³ CFU/ mg, while after treatment with ECMCRA the OSC ranged from 0–3.2×10³ CFU/mg.

Comparison of the methods by the Student T-Test showed that they are comparable, since there were no significant differences between them (p=0.09). From 35 teeth included in the analysis, 28(80%) no longer presented the Oral Streptococcus after treatment by Carisolv gel, while in the ECMCRA one, the elimination of Oral Streptococcus was observed in 27 (77.1%) teeth Fig.(3) .

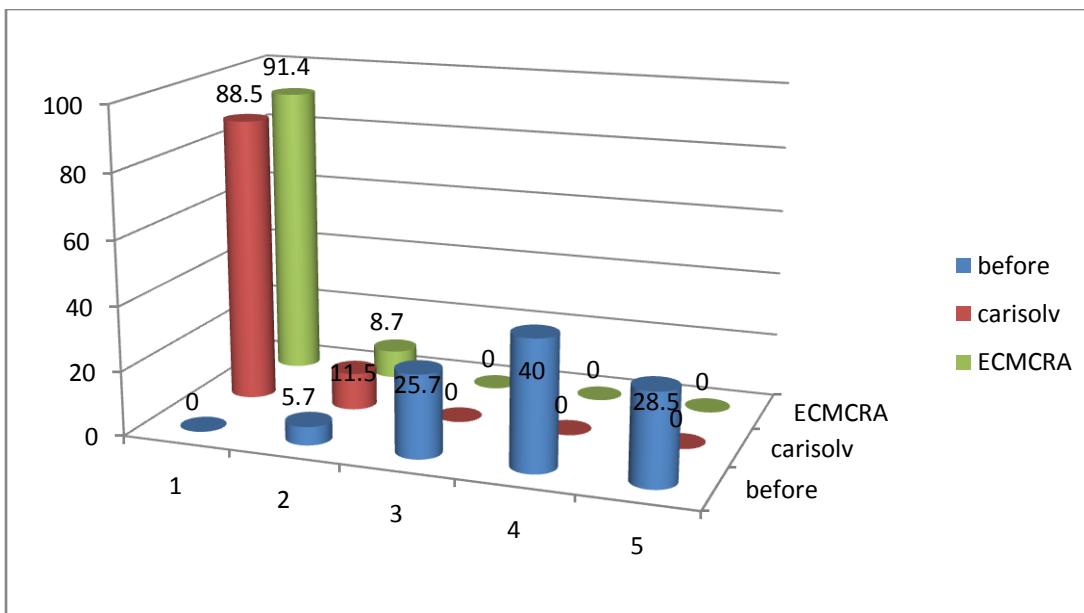


Figure (3): Percentage of Oral Streptococcus spp. count of carious lesions before and after treatment with Carisolv and ECMCRA in class 1 $\leq 10^2$; 2:10 3 ; 3:1001-10 4 ; 4:10 001-10 5 ; 5:100 001-10 6 ; 6: $>10^6$.

For Oral Lactobacillus spp. in CFU/mg the carious dentine counts ranging from 6.5×10^3 - 5.4×10^7 CFU/mg before treatment. After treatment with Carisolv[®], the dentine presented counts ranging from 0- 2.3×10^3 CFU/mg. In ECMCRA treated dentine the count range from 0- 3.1×10^4 CFU/mg after treatment.

From 35 teeth included in the analysis, 22(62.8%) no longer presented the Oral Lactobacillus spp. after treatment by Carisolv method, while in the ECMCRA one the elimination of Oral Lactobacillus spp. was observed in 21(60%) teeth.

Comparison of the two methods by the Student T-Test showed that there was no significant difference ($p > 0.05$) for Oral Lactobacillus spp. After both treatment gels Fig. (4).

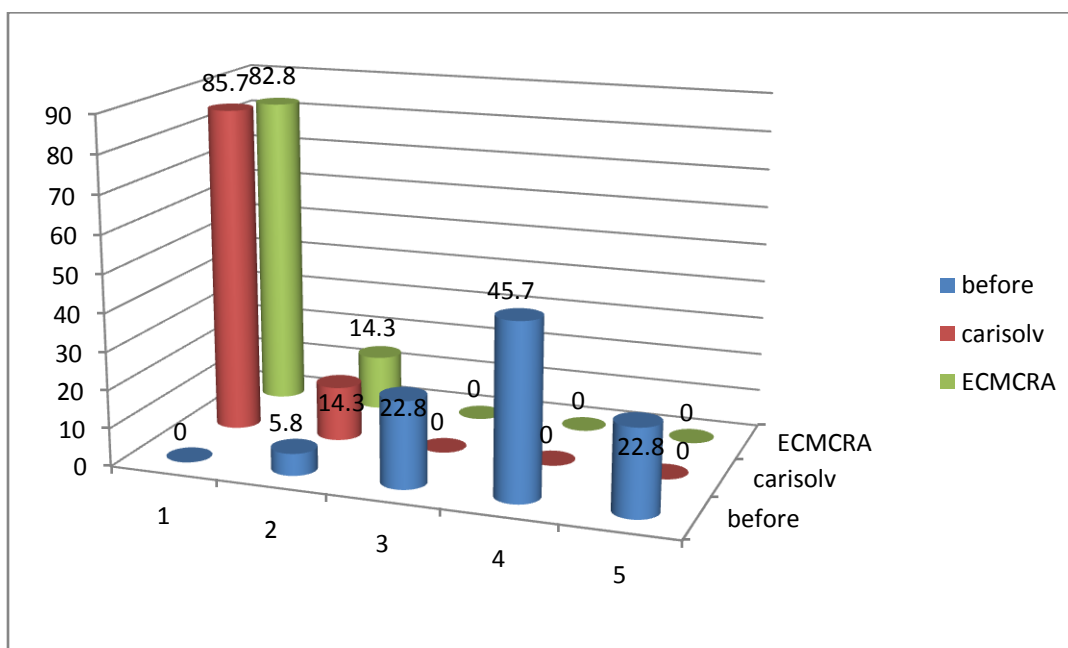


Figure (4): Percentage of Oral Lactobacillus spp. count of carious lesions before and after treatment with Carisolv and ECMCRA in class 1 $\leq 10^2$; 2:10 3 ; 3:1001-10 4 ; 4:10 001-10 5 ; 5: $>10^6$.

DISCUSSION

It is a well-established fact that bacteria are the prime etiological factor in the development and progression of dental caries. Two bacterial genera are of special interest in cariogenesis namely the mutans streptococci (MS) and lactobacilli^(19,20).

A chemomechanical caries removing system, acts by causing further degradation of the partially degraded collagen, in the infected dentine⁽²¹⁾. In contrary, the use of rotary instruments in the conventional mechanical method most of the time involves the removal of healthy dental tissue. This is not considered satisfactory, because there is an over-removal of the dental tissue softened by the demineralization that precedes the bacterial invasion which would be able to be mineralized⁽²²⁾.

Our study shows that carious dentine showed significantly higher bacterial cfu/mg counts and more samples contained total bacteria, oral Strep. spp and oral lactobacillus spp. counts than non-carious dentine did. Similar findings have been reported^(23,24).

A significant reduction in both the total bacterial count, oral Strep. spp. and oral lactobacillus spp. count was observed. Comparison of the two methods from a bacteriological point of view showed that the total bacterial count was reduced to less than 10^3 CFU in 82.8% of the samples after treatment with Carisolv and in 89.5% of the samples after the application of ECMCRA. This is in accordance with studies that reported a similar reduction in bacterial counts following the use of CMCR^(24,25,26). Also, our results coordinate with the other studies which inferred Papacarie as an excellent option for minimally invasive removal of caries tissue, achieving significant reduction in total bacteria and Streptococcus mutans^(27,28).

The antimicrobial effect of NaOCl presented in the composition of Carisolv results from the formation of hypochlorous acid (HOCl), when in contact with organic debris HOCl exerts its effect by oxidation of sulphhydryl groups within bacterial enzyme systems, thereby disrupting the microbial metabolism system⁽²⁹⁾.

Antibacterial activity of papain presented in concentration of 10% could be correlated to its antioxidation and scavenging action on superoxide and hydroxyl radical, which could be part of cellular metabolism of the bacteria⁽³⁰⁾.

Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc.⁽³¹⁾. Flavonoids presented in papain are hydroxylated phenolic substances known to be synthesized by plants, they have been found to be antimicrobial substances against a wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall^(32,33).

In this comparative study, the two methods of caries removal proved to be similar in their capacity of reducing Oral Lactobacillus spp. levels, and both chemomechanical methods had a more significant effect on the Oral Streptococcus spp. counts by apparently eliminating this microorganism in many cases.

The possible two explanation, first might be due to the antimicrobial action of sodium hypochlorite in Carisolv and papain and chloramine present in ECMCRA. Secondly, may be related to the evolution of the cariogenic process, which is generally initiated by Oral Streptococcus spp., but progression of the disease is associated with other cariogenic bacteria, including Oral Lactobacillus spp. According to Van Stripp, et al.,(1997)⁽³⁴⁾, Lactobacillus spp. are positively correlated with advanced lesions of carious dentine. Thus, in these situations, most Oral Lactobacillus spp. bacteria would be protected in an inner location in the carious dentine, and the antimicrobial action of both gel influences the elimination of this group of microorganisms to a lesser extent than Oral Streptococcus spp. The latter microorganism, in turn, would be more exposed to the antimicrobial action of chemomechanical agents, but this does not assure that the excavated tooth is bacteria-free or, at least, that a significant reduction in bacterial colonization has occurred.

While Oral Streptococcus spp. are mainly implicated with the initiation of enamel caries, they are only rarely the predominant species isolated from carious dentine. Instead, the composition of the microflora is known to become more complex as the lesions progress, and obligate anaerobes, mainly Gram-positive rods belonging to the genera become the predominant cultivable organisms in carious lesion⁽³⁵⁾.

Clinically, there are not any perfect clinical criteria to determine whether a cavity is caries-free or not. The useful methods nowadays are such as dentine acoustic and optical criteria, structure and moisture of dentine are very subjective⁽¹⁰⁾. But when we used CMCR an additional clue about caries removal criteria could be obtained by the clouding of the applied gel

is given as a caries indicator by the manufacturer. The manufacturer is judged a cavity to be caries-free when the gel no longer turns cloudy with debris.

Until nowadays, the clinical impact of bacterial persistence in caries-free dentine is not clear, but some authors agree that elevated bacterial counts remaining after a caries removal procedure can be considered clinically significant because they cause further disease progression⁽³⁶⁾, while, several investigations could show that often a low number of residual microorganisms (10^1 - 10^3 CFU/mg) remains behind in clinically sound hard dentine in spite of a significant reduction in the bacterial count; however, this low number of bacteria is considered to be clinically acceptable by several authors^(36,37,38).

Residual bacteria cannot be held solely responsible for occurrence of secondary caries, since individual factors like oral hygiene and dietary habits of the patients also have a profound influence on caries progression. More important is the quality of the filling covering the infected dentine. What is clear is that leaking fillings represent a caries risk⁽¹²⁾. Both Heinrich et al., (1991)⁽³⁹⁾ and Kidd et al. (1996)⁽³⁷⁾ studied the relationship of the clinical appearance of carious dentine and the number of bacteria, and they found, as in this study, values below 10^3 CFU for the total bacterial count for streptococci and lactobacilli in hard dentine.

The small amount of residual microorganisms remains negligible because it does not exceed the clinically accepted level. Moreover, with the new adhesive restorations providing perfect sealed margins and with the recently introduced antimicrobial cavity cleaners, this touch extent of demineralization bacteria can be minimized, all this provide a good evidence for acceptance of such value of bacteria^(40,41).

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