Partial Caries Removal in Primary Teeth: Association of Clinical Parameters with Microbiological Status

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Abstract

The relationship between clinical characteristics of carious dentin and bacterial colonization after partial caries removal is not completely understood. The aim of this study was to compare microbial counts between categories of carious dentin color, consistency and humidity, and to evaluate the correlation between these characteristics and the presence of cariogenic microorganisms in deep cavities (2/3 or more of the dentin thickness) submitted to partial caries removal. Sixteen primary teeth were submitted to the removal of all carious tissue from the lateral walls of the cavity, whereas carious tissue of the pulp wall was removed superficially. Dentin in the pulp wall was classified according to color, consistency and humidity immediately after cavity preparation and 3–6 months after cavity sealing and a tissue sample was collected on the same occasion for microbiological evaluation. Before sealing, \textit{Streptococcus mutans} (p = 0.033) and \textit{Lactobacillus} spp. (p = 0.048) counts were higher in cavities with humid dentin compared to cavities with dry dentin. A negative correlation was observed between carious dentin consistency and \textit{S. mutans} count during this phase ($r_s = -0.571$; $p = 0.020$). Arrest of dentinal caries lesions was observed after sealing, which was characterized by a reduction of bacterial counts and changes in dentin color, consistency and humidity, irrespectively of baseline dentin characteristics. The clinical characteristics of carious dentin change after the period of cavity sealing and cannot be applied as absolute indicators to limit the excavation of carious dentin when minimally invasive techniques are used.

Key Words
Clinical trial · Dental caries · Dental cavity preparation · Microbiology

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Removal of carious tissue during conventional cavity preparation is guided by clinical criteria of color, consistency and humidity [Kidd et al., 1993]. Although subjective, these criteria are traditionally used to determine the time when to interrupt the removal of demineralized dentin [Banerjee et al., 2000].

At present, minimally invasive techniques are recommended for the treatment of deep caries lesions in order to preserve pulp vitality and to prevent caries progression [Tyas et al., 2000; Mount, 2007; Bjørndal et al., 2010]. A deep caries lesion may be defined as a lesion where the demineralized dentin penetrated 2/3 [Gruythuysen et al., 2010] or 3/4 [Bjørndal and Kidd, 2005] or more of the en-
tire dentin thickness radiographically. Within this context, the objective of partial caries removal is to eliminate only superficial carious dentin that is highly infected, whereas dentin able to remineralize is maintained [Kidd, 2004].

In the case of partial caries removal, the differentiation between dentin to be removed and the dentin that will remain on the cavity floor should be done based on criteria of consistency and tissue texture [Massara et al., 2002]. However, the relationship between dentin consistency and the presence of microorganisms in the cavity during partial caries removal is controversial. In this respect, some investigators observed no association between dentin consistency and bacterial colonization [Böneck et al., 2003; Maltz et al., 2007] whereas others showed that the softer the dentin, the larger the number of _Streptococcus mutans_ and _Lactobacillus_ ssp. [Ayna et al., 2003; Orhan et al., 2008].

With respect to dentin color, some studies suggest that this parameter is not an absolute indicator of the presence of microorganisms during partial caries removal [Maltz et al., 2007; Orhan et al., 2008]. However, microbiological analysis at the end of cavity preparation showed that darker cavities contained larger numbers of _S. mutans_ and _Lactobacillus_ ssp. [Bjørndal et al., 1997; Ayna et al., 2003].

The association between humidity and dentin colonization by microorganisms before and after sealing of cavities submitted to minimally invasive preparation is unknown, although studies have shown that dentin becomes drier after a period of cavity sealing [Bjørndal et al., 1997; Orhan et al., 2008].

Therefore, since the relationship between clinical characteristics of carious dentin after partial caries removal (color, consistency and humidity) and bacterial growth is not completely established, the objective of the present study was to evaluate the relationship between these clinical characteristics and colonization with cariogenic microorganisms in cavities submitted to partial caries removal before and after a period of cavity sealing.

**Subjects and Methods**

**Sample Selection and Inclusion and Exclusion Criteria**

The study was approved by the Ethics Committee of the University Hospital of the Federal University of Maranhão (HU-UFMA, protocol 380/06). The parents or legal guardians of the children were informed about the objectives of the study and signed a free informed consent form, permitting the participation of minors. All children received instructions regarding oral hygiene and underwent dental treatment at the Pediatric Dentistry Clinic of UFMA.

The sample size was calculated based on a pilot study involving 6 teeth. Sixteen teeth were calculated to be necessary to obtain a power of the test of 80% at a level of significance of 5%, with a maximum error of the estimate of 0.6 colony-forming units (CFU, logarithmically transformed).

A sample was selected among children seeking treatment at the Pediatric Dentistry Clinic of UFMA. Each patient was submitted to initial clinical and radiographic examination for selection of the sample (fig. 1). Criteria for inclusion in the study were: (a) an active deep caries lesion in the inner portion of dentin (2/3 or more of the dentin thickness) of a primary molar that had not been restored previously and that involved the occlusal or occlusoproximal surface. All lesions should be cavitated with the exposure of the dentin and classified with a code 6 according to the International Caries Detection and Assessment System [Ekstrand et al., 2007]; (b) no clinical signs of irreversible damage to pulp such as spontaneous pain, fistula, and marked tooth mobility associated with soft tissue alterations; (c) no radiographic alterations in the interradicular or periapical region. Children with systemic disease or those using medications that may interfere with etiological factors of caries disease were excluded. Sixteen teeth were selected from children aged 5–8 years.

**Clinical Procedures**

The teeth of the patients were anesthetized and isolated with a rubber dam. A high-speed bur was used to gain access to the caries lesion. Cavity preparation was limited to the removal of carious tissue from the cavosurface angle and surrounding walls. Only superficial carious dentin was removed from the pulp wall with smooth spherical burs and manual instruments. On this occasion, excavation was limited to small dentin fragments that detached upon light passage of a dental curette, with deeper carious dentin being maintained.

**Clinical Evaluation and Collection of Dentin Samples**

After cavity preparation, cavity floor dentin was classified according to the modified criteria of Bjørndal et al. [1997]. Color was classified as yellow, light brown and dark brown. Consistency was classified as soft (probe penetrating dentin with no resistance when the probe is removed), medium hard (slight resistance when the probe is removed), and hard (resistance comparable to healthy dentin). Dentin was classified as wet or dry according to the presence or absence of humidity after mild drying of the cavity for 3 s. This modified criterion was used because we did not find any light-yellow or black dentin during the pilot study and the differentiation between very soft and soft dentin was difficult to achieve. Clinical evaluation was performed by a single examiner calibrated during the pilot study (C.C.C.R.). Calibration was performed at baseline and after 3 months, resulting in kappa values of 0.788 (variable color), 0.847 (variable consistency) and 0.875 (variable humidity).

Next, antisepsis of the rubber dam was performed with 2% chlorhexidine solution and a sample of remnant dentin was collected with a low-speed sterile No. 3 bur to evaluate the microbiological status of the dental tissue [Kidd et al., 1993]. The position of the collected sample was recorded on a clinical chart to prevent subsequent sampling from the same site as described by Paddick et al. [2005]. The two samples were collected from the central caries.
ious dentine in the pulpal wall; one sample was collected from the buccal portion and the other from the lingual portion.

The bur containing the dentin sample was immediately transferred to a flask containing 1.5 ml thioglycolate and glass beads. The flask was kept on ice and sent to the Laboratory of Microbiology for processing. Dentin weight was calculated by the difference between the weight of the whole set (tube, glass pearls, culture medium and bur with dentin) and the previously determined weight of the set without dentin. Bur weight was measured before sterilization and recorded.

All teeth were protected with calcium hydroxide cement (Dycal, Dentsply, Rio de Janeiro, Brazil) and restored using an adhesive system and resin composite (Single Bond, Filtek Z 250, 3M, Sumaré, São Paulo, Brazil) according to manufacturer instructions.

A new radiograph was obtained 3–6 months after treatment for evaluation of the interradicular and periapical region and clinical examination was repeated to analyze signs and symptoms of pulp normality. The restorative and protective materials were then removed and dentin color, consistency and humidity were again evaluated. The second dentin sample was collected at a site different from the first sample to evaluate bacterial growth after the period of cavity sealing.

**Microbiological Procedures**

The flasks containing the dentin samples were shaken in a vortex (AP 56, Phoenix, Araraquara, São Paulo, Brazil) for 30 s to disperse bacterial aggregates and decimal dilutions were prepared in sterile saline (0.9% NaCl). Aliquots of 0.1 ml of each dilution were then spread onto plates containing Rogosa agar (Difco, BD Diagnostic Systems, Sparks, Md., USA) for *Lactobacillus* spp. counts [Rogosa et al., 1951] and Mitis Salivarius agar (Difco) supplemented with 20% sucrose (Difco), 0.2 units/ml bacitracin (Sigma-Aldrich, St. Louis, Mo., USA), and 1% potassium tellurite (Sigma) for *S. mutans* counts (MSB agar) [Gold et al., 1973]. Agar plates were incubated under anaerobic conditions for 48 h (Rogosa agar) or in a 5% CO₂ atmosphere for 48 h (MSB agar), as described by Jurgensen and Jurgensen [1982]. All bacterial counts were done in duplicate. After incubation, agar plates containing between 10 and 300 colonies were used in the determination of the colony-forming units per milligram, which were calculated by multiplying the number of colonies on the plate by the inverse of the dilution used, multiplied by the correction factor. The detection limit was 100 CFU/mg, considering the mean weight of dentin samples and the growth of one bacterial colony in the first dilution.

Two to three typical colonies of each culture medium were selected for the evaluation of cell morphology by Gram staining and biochemical tests were performed for the identification of the bacterial isolates [Sneath et al., 1986].

**Statistical Analysis**

Since the data showed no normal distribution (Shapiro-Wilk normality test), they were logarithmically transformed by the Box-Cox method. The clinical characteristics of dentin before and after cavity sealing were compared by Fisher’s exact test. Bacterial counts in colony-forming units per milligram were transformed in $\log_{10}$. The Kruskal-Wallis test was used to compare *S. mutans* and *Lactobacillus* spp. counts between categories of the variables color and consistency and the Mann-Whitney test was used for the variable humidity. Spearman’s correlation coefficient ($r_s$) was used to determine the correlation between initial and final clinical characteristics of dentin and microorganism counts. A level of significance of 5% was adopted. Analyses were performed using the Stata 9.1 software (Stata, Tex., USA).

**Results**

Before cavity sealing, most dentin samples presented a light brown color. After cavity reopening, the dentin was found to be darker, with this difference being significant ($p = 0.006$). With respect to dentin consistency, most cavities presented a medium hard consistency before sealing, whereas hard dentin was mainly observed after sealing, with a significant difference before and after sealing ($p = 0.002$). Half the cavities presented wet remnant dentin after partial caries removal, whereas dry dentin was observed in all cavities after 3–6 months of sealing ($p = 0.002$).

Before cavity sealing, no significant difference in *S. mutans* or *Lactobacillus* spp. counts were observed between the three categories of dentin color. Similarly, there was no difference in bacterial counts between the three categories of consistency. With respect to humidity, higher *S. mutans* and *Lactobacillus* spp. counts were observed in cavities with wet dentin before sealing compared to cavities with dry dentin (tables 1, 2).

A significant negative correlation between dentin consistency and the presence of *S. mutans* ($r_s = -0.571; p = 0.020$) was observed before cavity sealing, indicating that the higher the consistency of dentin, the lower the colonization with *S. mutans*. On the other hand, there was no
significant correlation between dentin consistency and the presence of *Lactobacillus* spp. \((r_s = -0.474; p = 0.063)\). Dentin color showed no significant correlation with consistency \((p = 0.816)\), or colonization with *S. mutans* \((p = 0.883)\) or *Lactobacillus* spp. \((p = 0.423)\) (table 3).

A significant reduction in *S. mutans* and elimination of *Lactobacillus* spp. were observed after cavity sealing, but no significant correlation could be established between the clinical parameters and presence of these microorganisms.

**Discussion**

Minimally invasive techniques have been recommended for the treatment of deep caries lesions as an alternative to complete caries removal [Ricketts et al., 2006; Thompson et al., 2008; Lula et al., 2009; Bjorndal et al., 2010]. In the minimally invasive approach, the accepted criterion to limit the excavation of carious tissue is the removal of clearly necrotic tissue and excavation is interrupted when dentin chips or flakes off [Massara et al., 2002]. However, no consensus exists regarding the association between this criterion and the presence of microorganisms. The present study showed that the lower the consistency of the dentin maintained after partial caries removal, the larger the number of *S. mutans*. This finding agrees in part with the studies of Ayna et al. [2003] and Orhan et al. [2008], who showed that soft dentin exhibits higher bacterial activity. However, no correlation between dentin consistency and *S. mutans* or *Lactobacillus* spp. count was observed after cavity sealing.

This finding indicates that the criterion of consistency may not be accurate to identify dentin that needs to be removed, since dentin will become harder irrespective of its initial consistency.

Dentin color was not an adequate parameter to differentiate the degree of infection before and after sealing, in
agreement with another microbiological study [Orhan et al., 2008]. One explanation for this finding would be that a color change is due to reduced cariogenic activity of microorganisms, bacterial degeneration and the pulp response in deep cavities [Malone et al., 1966; Di Nicolò et al., 2000] and is not related to the presence of specific microorganisms [Bjørndal and Larsen, 2000]. Additionally, color changes precede bacterial invasion in acute carious lesions [Fusayama et al., 1966]. Thus, the presence of dark dentin at the cavity floor during partial caries removal does not imply its excavation.

Another finding of the present study was that wet dentin harbors a larger number of cariogenic microorganisms than dry dentin, in agreement with the studies of Ayna et al. [2003] and Orhan et al. [2008]. However, dry dentin, the absence of Lactobacillus spp. and lower S. mutans counts were observed in all cavities after 3–6 months of cavity sealing, findings indicating the arrest of the caries process and the possibility to maintain visibly wet dentin at the cavity floor to prevent pulp exposure.

One question that cannot be overlooked is the possibility of misinterpretation of microbiological data due to large variation in bacterial growth. The measure of accuracy prediction used in the present study \(0.6 \log_{10} (CFU)\) had low influence on our results, even for the dry dentin category, in which mean growth was around \(1.2 \log_{10} (CFU)\) (tables 1, 2). In this sense, the difference between the real mean and the one reported was clinically not significant.

The present results support previous findings that some clinical parameters such as humidity and consistency may indicate the presence of cariogenic microorganisms immediately after partial caries removal [Kidd et al., 1993; Bjørndal et al., 1997]. However, typical changes characteristic of caries inactivation occur in dentin after cavity sealing [Bjørndal et al., 1997; Maltz et al., 2007]. Therefore, these clinical parameters cannot be applied as absolute indicators to limit dentin excavation when a minimally invasive approach is used [Bönecker et al., 2003].

Taken together, two aspects should be considered when the technique of partial caries removal is used: the correct diagnosis of pulp condition [Ricketts, 2001] and the maintenance of adequate cavity sealing [Ribeiro et al., 1999]. If pulp vitality is maintained irrespective of the initial characteristics of dentin, partial caries removal permits the formation of reparative dentin and dentinal sclerosis [Bjørndal, 2001]. In addition, maintenance of the cavity sealing through a dentinoenamel junction free from caries and adhesive restorations interrupts the communication of bacteria present in dentin with the surface biofilm, with a consequent reduction in nutrients and bacterial viability [Kidd, 2004]. These changes have been demonstrated in longitudinal studies of teeth submitted to partial caries removal [Mertz-Fairhurst et al., 1998; Ribeiro et al., 1999; Casagrande et al., 2009; Alves et al., 2010] and support the use of this technique for the one-visit treatment of deep caries lesions.

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**Disclosure Statement**

The authors declare that they have no conflict of interest.

**References**


