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Microbial Load After Selective and Complete Caries Removal in Permanent Molars: A Randomized Clinical Trial

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The aim of this randomized clinical trial was to compare the remaining microbial load after treatments based on complete and selective caries removal and sealing. Patients with active carious lesions in a permanent molar were randomly allocated into 2 groups: a test group (selective caries removal–SCR; n=18) and a control group (complete caries removal – CCR; n=18). Dentin samples were collected following the excavation and three months after sealing. Streptococcus species, *Streptococcus mutans*, Lactobacillus species, and total viable microorganisms were cultured to count the viable cells and frequency of species isolation. CCR resulted in significant lower total viable microorganisms counts ($p \leq 0.001$), Streptococcus species ($p \leq 0.001$) and Lactobacillus species ($p \leq 0.001$) initially. However, after sealing, a decrease in total viable microorganisms, Streptococcus species, and Lactobacillus species in the SCR resulted in no difference between the groups after 3 months. In conclusion, selective caries removal is as effective as complete caries removal in reducing dentin bacterial load 3 months after sealing.

Key Words: dentinal caries, microbiology, permanent molars, randomized clinical trial, selective caries removal.

Introduction

The presence of microorganisms in and inactive non-cavitated lesions shows that bacteria inside dental tissue (enamel and dentin) do not inhibit the arrestment of the caries process (1). Dentin contamination is increased after surface breakdown in cavitated lesions and the conventional restorative treatment for those lesions involves complete removal of the infected tissue based on the hardness criteria (2). The aim of this procedure is to ensure the treatment success via the elimination of bacteria and to provide a proper sealing to avoid caries progression (3). However, complete caries removal (CCR) does not make the excavated cavity free of bacteria (4,5), and the importance of the residual bacteria to caries progression remains under debate. Furthermore, in deep caries lesions, there is a high risk of pulp exposure by complete caries removal, compromising the success rate of the treatment (2,6-9). In order to preserve dental structures as much as possible, and to avoid irreversible damage to the pulp, some conservative techniques based on selective carious dentin removal (SCR) in deep carious lesions have been proposed (2,10,11).

It has been shown that caries lesions become arrested after partial excavation if they are isolated from the oral cavity (2), due to the limited carbohydrate supply from the diet. These residual microbiota under the filling are exposed to more homogeneity of the nutrients, primarily serum proteins instead of carbohydrates (12). This starvation stress significantly affects the remaining microorganisms, decreasing microbial load (2,8,10,13); species composition (10,12); and the genotypic diversity (12,14). Although acceptable clinical results have been observed after SCR, the persistence of viable bacteria in dentin has raised doubts regarding the long-term effectiveness of this treatment, especially after the concept of a tissue-dependent hypothesis for caries has been proposed, suggesting that caries can progress through dentin tissue even with limited access to dietary sugars (15).

A recent study evaluated bacterial levels after SCR and sealing (6 months) and showed that the microbial load was lower than after complete caries removal before sealing, but no data is shown comparing the presence of bacteria after sealing between the two treatments (16). Besides that, only one study has evaluated the residual contamination after a follow-up period, comparing CCR and SCR with a proper control group, although it was developed in deciduous teeth and detected a very unexpected result: an increase in the total microbial load after complete caries removal and sealing in a variable follow up period (3 to 6 months) (5). In a recent meta-analysis, it was shown that SCR appears advantageous compared with CCR. However, the authors concluded that evidence levels are currently insufficient for definitive conclusions because of high risk of bias within studies (11).

Many microbiological studies of cavities treated by SCR do not include a CCR control group. Furthermore, it is uncertain whether bacteria that remain after sealing of the cavity are able to proliferate, regardless of the

technique used for caries removal. Therefore, the aim of this randomized clinical trial was to compare the residual microbial load (total anaerobic, Streptococcus species and Lactobacillus species) after complete and selective caries removal and cavity sealing. The hypothesis is that there are no differences in microbial loads in both treatments.

Material and Methods

Trial Design

A double-blind, randomized, controlled clinical trial was designed to test the effect of complete (control group) versus selective (test group) caries removal on the microbial load in permanent molars by culture approaches. The study was approved by the ethics committee of the Federal University of Rio Grande do Sul (UFRGS), according to the Helsinki Declaration of 1975. The participants, aged 9 to 31 years, or their parents/legal guardians received detailed information describing the study and signed a free informed consent form. All the participants were recruited and received dental care at the Faculty of Dentistry, UFRGS. The clinical trial (including the follow-up period) was performed from August 2010 to August 2011.

Sample size calculations showed that 15 patients were needed in each group to detect a 0.25 standard deviation difference in the log count between selective and complete excavation (16) at a 2-sided alpha level of 5% and 80% power (http://www.lee.dante.br). A drop out rate of up to 20% was included; yielding a final sample size of 18 participants to be recruited per group. One tooth was treated in each randomized patient, totaling 36 cases.

Eligibility Criteria for Participants

The participants were required to fulfill the following eligibility criteria: presence of a permanent molar with active primary occlusal caries lesions located in the middle third of the dentin (detected through the interproximal radiographic examination) (Fig. 1), complete root formation, as well as symptoms and clinical signs associated with pulp tissue without irreversible alterations (positive response to cold test, absence of pain on percussion, and absence of spontaneous pain). The exclusion criteria were radiographic signs consistent with pulp involvement (thickening of the periodontal ligament or periapical lesion) (17).

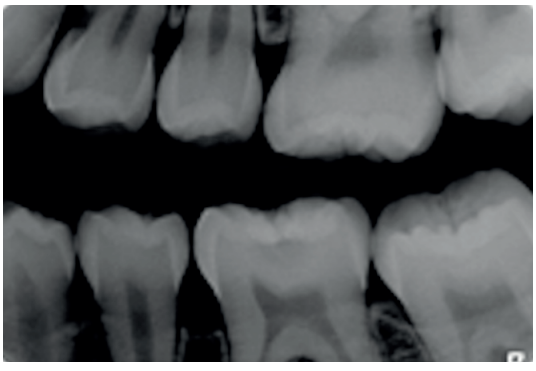

Figure 1. Radiographic image of an eligible molar (mandibular left first molar) to this study.

The allocation sequences for CCR (control) versus SCR (test) (1:1) were computer-generated (http://www.openepi.com/Menu/OpenEpiMenu.htm) in blocks of 10. The randomization was performed by only one researcher (V.K.S) and this information was only provided to the operator (L.B.F.) at the time of treatment, who was blinded until the caries removal procedure.

Interventions

Patients were submitted to dental prophylaxis, anesthesia and rubber dam isolation of the operative field. Antisepsis of the rubber dam was performed with 0.05% iodine alcohol before accessing the carious lesion (with a high-speed bur). The patient underwent an operation using one of the techniques for caries removal based on the clinical criteria of hardness, as previously described (17): a) complete caries removal - CCR or b) selective caries removal - SCR, with complete caries removal from surrounding walls and selective caries removal of the pulp wall by removing the necrotic and disorganized dentin layer. The cavity was washed with sterile saline and dried with sterile swabs. The lesions were conceptually divided into halves in a buccolingual direction. After dentin removal, a mesial dentin sample was taken for microbiological analysis. The bottom of the cavity was lined with a calcium hydroxide-containing base material (Dycal, Dentisply, Konstanz, Germany). The cavity was sealed with glass ionomer cement (Vitromolar, DFL, Rio de Janeiro, Brazil). Three months after sealing, patients were checked for clinical symptoms and positive response to the cold test (–20 °C refrigerated gas; Aero-jet, Rio de Janeiro, Brazil). The lesion was reopened and a dentin sample was taken from the distal half of the cavity. Glass ionomer base cement was placed on the floor of the cavity, and the tooth was restored with an adhesive system (3M Scotchbond Multi-Purpose, St. Paul, USA) and composite (Charisma, Kulzer, São Paulo, Brazil).

Outcome Measure: Microbiological Sampling

Dentin samples were collected by 2 sterile slowly-rotating no. 4 round burs (2,10). Before collection, the burs were moistened in a reduced transport fluid (RTF) medium to facilitate the impregnation of dentin in the bur. After collection, the dentin sample was immediately transferred to a sterile container containing 1.2 mL of RTF and glass beads . Samples were dispersed twice by sonication for 10 s, with a 30-s interval, in a high-density ultrasonic processor (Vibra-Cell, Sonics & Materials Inc., CT, USA), to disperse

bacterial aggregates. These were then vortexed for 30 s and serially diluted 10-fold in the RTF medium. Subsequently, 25 µL aliquots from the appropriate dilution were plated in duplicate on the following solid media: brain-heart infusion (BHI) agar (HiMedia, Mumbai, India) supplemented with 5% sheep blood and enriched with vitamin k-hemin for total viable microorganisms counts; mitis salivarius (MS) agar (Difco, BD, Sparks, USA) and 1% potassium tellurite for Streptococcus species; MS agar (Difco, BD, Sparks, USA) supplemented with 20% sucrose, 0.2 units/mL bacitracin, and 1% potassium telurite (MSB) for *S. mutans*; and Rogosa selective Lactobacillus agar (HiMedia, Mumbai, India) for Lactobacillus species. MS and MSB were incubated under microaerophilic conditions at 37°C for 48 h. Rogosa selective Lactobacillus agar was incubated anaerobically at 37 °C for 72 h. The BHI plates were incubated anaerobically at 37°C for 120 h. After incubation, the number of colony-forming units (CFUs) was determined. The counts derived from the selective media only included colonies with the relevant characteristic morphology. In case of doubt, 2 or 3 representative colonies from each culture medium were selected for Gram staining. A single blinded trained examiner performed the count.

Statistical Methods

The bacterial counts are expressed at log10. The constant 1 was added to the CFUs because many samples showed zero counts before and after the experimental period in both groups (2). A non-normal distribution was verified by Kolmogorov-Smirnov and Shapiro-Wilk tests. To compare the CFU counts before and after sealing in each group, the Wilcoxon signed rank test was used. The Mann-Whitney test was used to compare the CFU counts between the test and control groups. The McNemar test was used to determine whether there was any difference in the frequency of isolation before and after cavity sealing in each group. The chi-square test was used to compare the frequency of bacterial isolation before and after sealing between the 2 groups. The significance level was set at 5%. Bacterial counts in colony-forming units were analyzed in Stata 9.1 package (Stata, College Station, TX, USA). Other statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

From a total of 36 enrolled subjects, 2 were subsequently excluded from the analysis, both being from the test group (1 left the trial, and 1 had the temporary filling replaced by another dentist) (Fig. 2). Thus, the final sample consisted of 34 patients, 18 in the control group (CCR) and 16 in the test group (SCR). During the 3-month follow-up period, no patients had signs or symptoms consistent with pulp

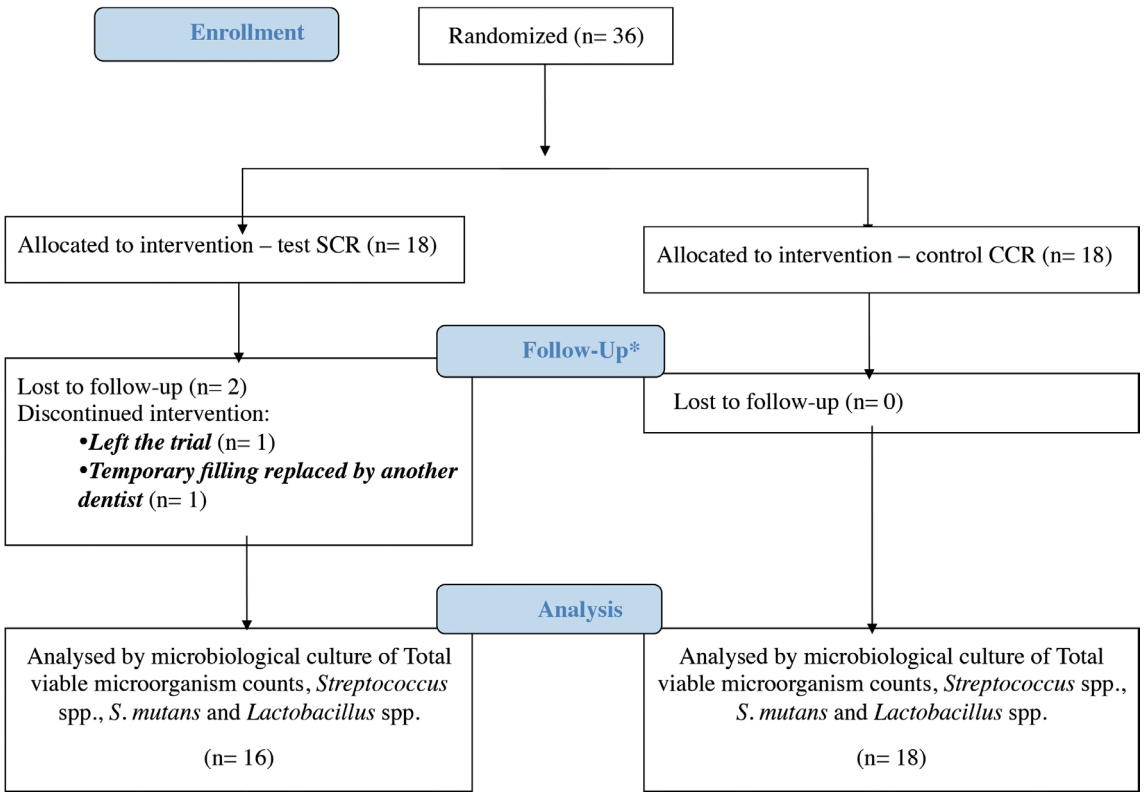

Figure 2. Flow Diagram of the study. *Follow-Up = the cavity was reopened after 3-month temporary sealing.

alteration. Initially, the bacterial counts before cavity sealing differed significantly between the 2 groups with respect to total viable microorganisms (p=0.02), Streptococcus species (p<0.001), and *S. mutans* counts (p=0.006). The Lactobacillus species counts did not differ significantly (p=0.08; Fig. 3).

There was no significant difference between the number of viable microorganism counts in the CCR group before and after sealing for any of the variables tested, although there was a trend toward a decreased count after sealing: total viable microorganisms, p=0.055; Streptococcus species, p=0.953; *S. mutans*, p=1.000; and Lactobacillus species, p=0.086. In the SCR group, significant reductions in the numbers of total viable microorganisms (p=0.002), Streptococcus species (p=0.003), and Lactobacillus species (p=0.006) were observed after sealing, but there was no significant reduction in the *S. mutans* counts after sealing (p=0.310; Fig. 3). Low *S. mutans* prevalence and counts were observed in the initial samples.

Three months after sealing, no statistically significant differences in counts were evident between the control and test groups for any of the microorganisms targeted in this study: Streptococcus species, p=0.237; *S. mutans*, p=0.551; Lactobacillus species, p=0.281; and total viable microbial load, p=0.109 (Fig. 3).

The number of samples exhibiting detectable bacterial growth before and after sealing decreased only in the

Table 1. Number of sample with bacterial growth submitted to complete caries removal (CCR) and selective caries removal (SCR) before and after sealing

		Initial	Final	p
Total viable microorganisms counts	CCR	12	10	0.68
	SCR	16	11	0.06
	p	0.02*	0.49	
Streptococcus spp.	CCR	7	7	1
	SCR	16	8	0.007*
	p	<0.001*	0.73	
Lactobacillus spp.	CCR	9	4	0.125
	SCR	13	6	0.04*
	p	0.08	0.457	
S. mutans	CCR	0	0	ND
	SCR	6	2	0.21
	p	0.006*	0.21	

ND: not detected. "Initial"= Before temporary cavity sealing; "Final" = After temporary cavity sealing. * p<0.05

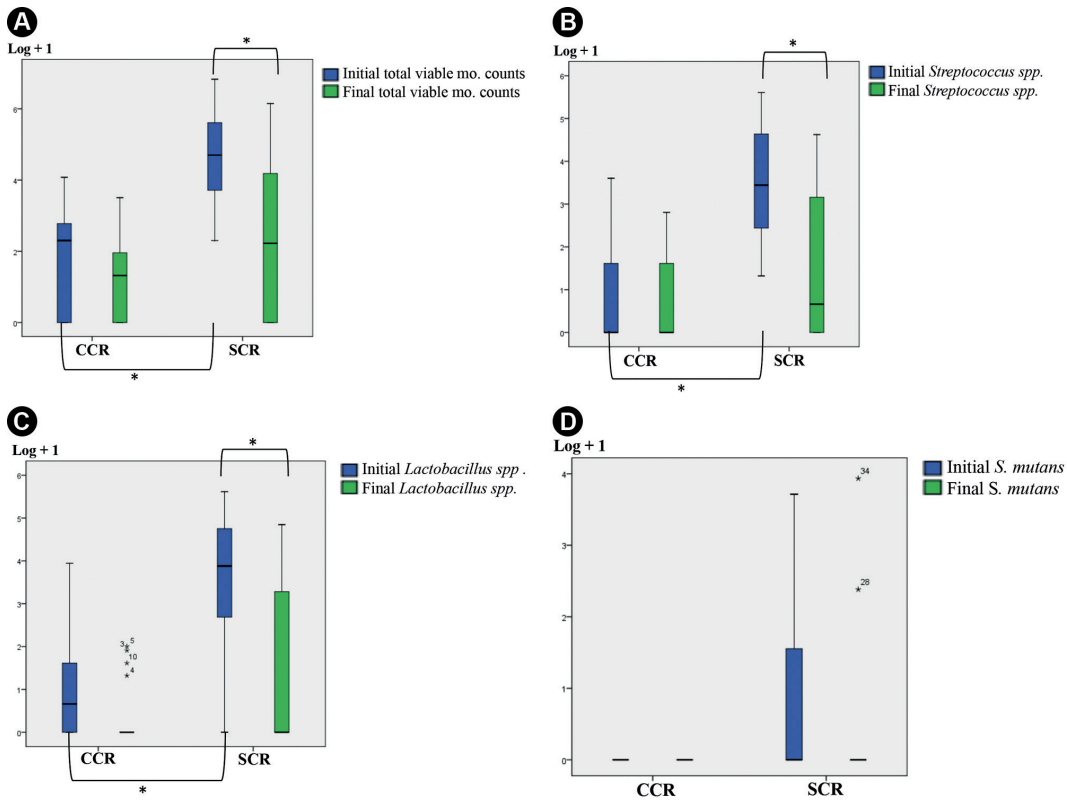

Figure 3. Median and interquartile range of the number of selected bacteria in log10 + 1 (CFU) of dentin before ("initial") and after ("final") cavity sealing in patients submitted to complete caries removal (CCR) or selective caries removal (SCR). A: Total viable microorganisms (mo.) counts; B: Streptococcus spp. counts; C: Lactobacillus spp. counts; D: *S. mutans* counts. * p<0.05

selective caries removal group, with regard to Streptococcus (p=0.008) and Lactobacillus species (p=0.039). After sealing, no differences were detected between the CCR and SCR groups for any of the cultured species (Table 1).

Discussion

There is continued interest in determining whether there is a real need to remove all carious dentin before filling. This randomized clinical trial comparing complete and selective caries tissue removal showed a similar microbial load in both treatments after three months, by analyzing the microbial load as a biomarker of the effectiveness of treatment. The results showed that SCR was as effective as CCR. The present study adds to the body of evidence implying that selective carious removal is acceptable for effective treatment of deep lesions. The depth of the lesions in the middle third of the dentin was selected to avoid the risk of pulp exposure during sample collection that would probably occur in deep carious lesions, leading to a bad tooth prognosis (9).

In SCR, microorganisms were evidently less prevalent after cavity sealing than before cavity sealing. This finding was also observed by previous studies that investigated only SCR and sealing (2,4,8,10,12). Furthermore, SCR and sealing were associated with decreased total viable microorganisms counts here, although the same number of lesions remained with these microorganisms at detectable levels, which differs from the results of a previous study in which higher total counts of microorganisms were observed after sealing (5). The higher counts reported by Lula et al. (5) were explained by them as a possible microinfiltration expected after resin composite restorations and the proliferation of bacteria that had adapted to this specific environment. In the present study, the decreasing number of microorganisms after the sealing period was similar when comparing CCR and SCR.

The prevalence of microorganisms in dentin after SCR has raised doubts regarding the long-term effectiveness of the treatment. Potential selection for cariogenic bacteria may lead to carious progression. In accordance with literature, *S. mutans* was detected in the carious dentin before sealing, which was related to the progression of the carious process (12). In carious lesions, the pH is subject to large perturbations dependent upon the consumption of fermentable carbohydrates, which facilitate rapid acid production and consequent establishment of an acidic environment (12). This acidic environment results from the presence of acidogenic and aciduric bacteria such as lactobacilli and *S. mutans*. After sealing, cariogenic microorganisms such as Lactobacillus species and *S. mutans* were barely detectable. This phenomenon was also observed in another study that compared the effects of sealing infected carious dentin beneath restorations by conventional isolation and enumeration methods (10). Only non-mutans streptococci and gram-positive pleomorphic rods were detected in the final samples in the study by Paddick et al. (12). Viable streptococci were detected in the final sample after SCR and CCR, but they were not found in higher quantities when compared to the initial samples, changing the proportion of species after sealing.

The hypothesis of the reduction of microbial loads was confirmed, as biofilm access to exogenous nutrients is blocked in this context, creating a selective environment for microorganisms in which only those most able to adapt will survive. The lack of external nutrients available to the microbiota growth is the most obvious environmental challenge for the remaining microorganisms (nutritional stress). Paddick et al. (12) have reported that only bacteria capable of producing enzymes that cleave the terminal sugars from glycoproteins were recovered from the dentin after cavity sealing. Moreover, it is interesting to note that in addition to external nutritional starvation, there is also a reduction of dentin permeability due to dentinal tubular sclerosis (12). It is also likely that components of the dead bacteria that did not survive after sealing may contribute to this phenomenon, acting as a source of nutrients to those bacteria that survive beneath the restorations (12).

Due to the limitations of cultured-based studies that underestimate non-cultivable microorganisms in oral biofilms, many DNA-based methods have been used to analyze carious biofilms. These molecular biology tools have contributed significantly in identifying the microbiota composition and diversity. However, it is impossible to determine by DNA-based methods if the viable bacteria are metabolically active as pathogens, or if they are only present in the environment due to their resistance to the inhospitable environment. The viability of the microbiota was essential to the results of the present study. Therefore the cultivable methods are still applicable here.

It is widely believed that if infected tissue remains, the carious process may continue, albeit slowly (18). Studies have shown that SCR in deep lesions is a promising alternative for preserving pulp vitality, showing clinical success even after 10 years (2,19). Here, the number of microorganisms was reduced after sealing the cavity, reaching the levels usually encountered in cavities in which all carious tissue is removed according to the hardness criteria (2,8,10,13,20). A study also showed that after SCR and sealing, the microbial load is less abundant than after CCR (16). The similarity of microbial infection evident after cavity sealing following CCR and SCR suggests that SCR could be a more conservative alternative with regard to the treatment of carious lesions. Based on the microbiological findings, reentry after SCR to remove all the remaining carious tissue (stepwise excavation) is unnecessary and may be potentially

detrimental in some circumstances.

There is only one study comparing the in vivo effect of different sealing materials in direct contact with permanent teeth dentin tissue (calcium hydroxide cement, glass ionomer cement and amorphous material) after a period of sealing (21). It showed no significant difference between the groups, and the sealing actually impacted the reduction of viable microorganisms. Thus, it does not seem that the results found in this study had an influence of the temporary sealing materials, but to the sealing period.

In conclusion, the similarity between the microorganism's counts observed after conventional excavation (CCR) compared with SCR suggests that there is no need to perform complete caries removal based on conventional clinical criteria of hardness. CCR may represent overtreatment in some instances, resulting in the unnecessary tissue removal.

Resumo

O objetivo deste ensaio clínico randomizado foi comparar os microrganismos remanescentes após tratamentos baseados em remoção total de tecido cariado e selamento e a remoção seletiva de tecido cariado e selamento. Pacientes com lesões de cárie ativas em molares permanentes foram divididos aleatoriamente em dois grupos: grupo teste (remoção seletiva de tecido cariado-SCR; n=18), e grupo de controle (remoção total de tecido cariado-CCR; n=18). Amostras de dentina foram obtidas após a remoção da tecido cariado e após 3 meses de selamento das cavidades. Streptococcus spp., *Streptococcus mutans*, Lactobacillus spp. e microrganismos viáveis totais foram cultivados para contagem de células e frequência de isolamento de espécies. CCR resultou em menores contagens totais de microorganismos viáveis (p≤0,001), Streptococcus spp. (p≤0,001) e Lactobacillus spp. (p≤0,001) inicialmente. Entretanto, após o selamento, uma redução significativa nas contagens totais de microrganismos viáveis, Streptococcus spp. e Lactobacillus spp. resultou em nenhuma diferença entre os grupos após 3 meses. Conclui-se que a remoção seletiva de cárie é tão seletiva quanto a remoção completa de cárie na redução da infecção dentinária após três meses com selamento da lesão.

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References

1. Parolo CC, Maltz M. Microbial contamination of noncavitated caries lesions: a scanning electron microscopic study. Caries Res 2006;40:536-541.
2. Maltz M, de Oliveira EF, Fontanella V, Bianchi R. A clinical, microbiologic, and radiographic study of deep caries lesions after incomplete caries removal. Quintessence Int 2002;33:151-159.
3. Brännström M, Gola G, Nordenvall KJ, Torstenson B. Invasion of microorganisms and some structural changes in incipient enamel caries. A scanning electron microscopic investigation. Caries Res 1980;14:276-284.
4. Orhan AI, Oz FT, Ozcelik B, Orhan K. A clinical and microbiological comparative study of deep carious lesion treatment in deciduous and young permanent molars. Clin Oral Investig 2008;12:369-378.
5. Lula EC, Monteiro-Neto V, Alves CM, Ribeiro CC. Microbiological analysis after complete or partial removal of carious dentin in primary teeth: a randomized clinical trial. Caries Res 2009;43:354-358.
6. Leksell E, Ridell K, Cvek M, Mejare I. Pulp exposure after stepwise versus direct complete excavation of deep carious lesions in young posterior permanent teeth. Endod Dent Traumatol 1996;12:192-196.
7. Ricketts DN, Kidd EA, Innes N, Clarkson J. Complete or ultraconservative removal of decayed tissue in unfilled teeth. Cochrane Database Syst Rev 2006;3:CD003808.
8. Bjorndal L, Larsen T, Thylstrup A. A clinical and microbiological study of deep carious lesions during stepwise excavation using long treatment intervals. Caries Res 1997;31:411-417.
9. Bjorndal L, Reit C, Bruun G, Markvart M, Kjaeldgaard M, Nasman P, et al. Treatment of deep caries lesions in adults: randomized clinical trials comparing stepwise vs. direct complete excavation, and direct pulp capping vs. partial pulpotomy. European journal of oral sciences 2010;118:290-297.
10. Bjørndal L, Larsen T. Changes in the cultivable flora in deep carious lesions following a stepwise excavation procedure. Caries Res 2000;34:502-508.
11. Schwendicke F, Paris S, Tu YK. Effects of using different criteria for caries removal: a systematic review and network meta-analysis. J Dent 2015;43:1-15.
12. Paddick JS, Brailsford SR, Kidd EA, Beighton D. Phenotypic and genotypic selection of microbiota surviving under dental restorations. Appl Environ Microbiol 2005;71:2467-2472.
13. Wambier DS, dos Santos FA, Guedes-Pinto AC, Jaeger RG, Simionato MR. Ultrastructural and microbiological analysis of the dentin layers affected by caries lesions in primary molars treated by minimal intervention. Pediatr Dent 2007;29:228-234.
14. Damé-Teixeira N, Arthur RA, Parolo CC, Maltz M. Genotypic diversity and virulence traits of *Streptococcus mutans* isolated from carious dentin after partial caries removal and sealing. Sci World J2014;2014:165201.
15. Simón-Soro A, Belda-Ferre P, Cabrera-Rubio R, Alcaraz LD, Mira A. A tissue-dependent hypothesis of dental caries. Caries Res 2013;47:591-600.
16. Maltz M, Henz SL, de Oliveira EF, Jardim JJ. Conventional caries removal and sealed caries in permanent teeth: a microbiological evaluation. J Dent 2012;40:776-782.
17. Maltz M, Jardim JJ, Mestrinho HD, Yamaguti PM, Podestá K, Moura MS, et al. Partial removal of carious dentine: a multicenter randomized controlled trial and 18-month follow-up results. Caries Res 2013;47:103-109.
18. Kidd EA. How 'clean' must a cavity be before restoration? Caries Res 2004;38:305-313.
19. Alves LS, Fontanella V, Damo AC, Ferreira de Oliveira E, Maltz M. Qualitative and quantitative radiographic assessment of sealed carious dentin: a 10-year prospective study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:135-141.
20. Pinto AS, de Araújo FB, Franzon R, Figueiredo MC, Henz S, García-Godoy F, et al. Clinical and microbiological effect of calcium hydroxide protection in indirect pulp capping in primary teeth. Am J Dent 2006;19:382-386.
21. Corralo DJ, Maltz M. Clinical and ultrastructural effects of different liners/restorative materials on deep carious dentin: a randomized clinical trial. Caries Res 2013;47:243-250.

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