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Micro-computerized tomography assessment of fluorescence aided caries excavation (FACE) technology: comparison with three other caries removal techniques

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ABSTRACT

Background: The aim of this study was to determine the caries removal effectiveness (CRE) and minimal invasiveness potential (MIP) of four dentine caries removal methods.
Methods: After carious molars were scanned using micro-computerized tomography (micro-CT), dentine caries were removed by fluorescence aided caries excavation (FACE) technology, laser induced fluorescence (LIF), chemomechanical excavation (CME), and conventional excavation (CE). Micro-CT was then repeated. CRE was determined based on the volume of residual caries/initial caries (RC/IC) and the mean mineral density (MD) at the cavity floor. MIP was determined by measuring the volume of the prepared cavity/initial cavity (PC/IC).
Results: Among the four groups, the LIF group had the smallest RC/IC (0.08), the highest mean MD at the cavity floor (1.32 g/cm^3) and the highest MIP (4.47). The CME group had the highest RC/IC (0.24), the lowest mean MD (1.01 g/cm^3) and the lowest MIP (2.23). The CE group exhibited a more acceptable CRE (RC/IC = 0.13, mean MD = 1.21 g/cm^3) but had a higher MIP (3.95). Both the CRE and MIP parameters of FACE technology were the second most acceptable (RC/IC = 0.12, mean MD = 1.13 g/cm^3, MIP = 3.20) and did not differ significantly from the most acceptable.
Conclusions: FACE is an effective caries removal technology for removing infected dentine without significantly increasing cavity size.

Keywords: Fluorescence aided caries excavation (FACE), dentine caries, caries excavation, Carisolv, DIAGNOdent, micro-computerized tomography.

Abbreviations and acronyms: ANOVA = analysis of variance; CE = conventional excavation; CME = chemomechanical excavation; CRE = caries removal efficacy; FACE = fluorescence aided caries excavation; LIF = laser induced fluorescence; LSD = least significant difference; MD = mineral density; micro-CT = micro-computerized tomography; MI = minimally invasive; MIP = minimal invasive potential; PC/IC = prepared cavity/initial cavity; RC/IC = residual caries to initial caries.

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INTRODUCTION

In recent years, the concept of minimally invasive (MI) dentistry has emerged. MI calls for the selective removal of heavily infected and irreversibly denatured dentine caused by carious lesions while preserving dentine that is demineralized but not infected.[1,2] Clinically, however, it is difficult to detect this boundary, which may lead to either under- or over-excavation.

Numerous caries excavation methods and techniques are available to dentists. Conventional excavation (CE) involves the use of a slow-speed tungsten carbide bur, and dentists determine the extent of the excavation by the hardness and colour of the dentine.[3] Clinicians commonly excavate until reaching the firm[4,5] dentine and rarely consider whether demineralized dentine could be preserved and might have the potential for remineralization.[6] Moreover, deciding on the depth of excavation based on the colour of the tissue and on hardness lacks a precise specification criterion.

Chemomechanical excavation (CME) of carious dentine has become an alternative to CE, particularly in paediatric dentistry[7] and for anxious or medically compromised patients.[8] This method disrupts the altered collagen fibres in carious dentine and allows a higher selectivity of removal. It also avoids the painful and excessive removal of healthy dentine.[7,9–12] A recent report[13] compared nine caries removal technologies and concluded that self-limited CME excavation using Carisolv (MediTeam, Goteborg, Sweden) and hand excavators was more selective in preserving the healthy tissue than other alternative methods.

Another caries removal method, laser induced fluorescence (LIF), uses the DIAGNOdent system (Kavo, Biberach, Germany) to differentiate between carious and sound tissue. Fluorescence is the emission of light by a substance that is previously excited by light or other electromagnetic radiation and is a form of luminescence.[14] The DIAGNOdent system quantifies the fluorescence intensity and converts it to a value within a scale ranging from 0 to 99 based on the fluorescence of a calibrated standard.[16] Healthy dental tissues exhibit lower fluorescent readings (0–12) and are used to define the endpoint of the intervention.[16]

A newer caries excavation method, fluorescence aided caries excavation (FACE), that uses orange-red autofluorescence as a marker for infected dentine was recently introduced.[17–20] The phenomenon of red fluorescence in carious dental hard tissue and in dental plaque and calculus was first reported in the 1920s.[22,23] This red autofluorescence of caries and dental plaque is believed to be caused by porphyrins and metalloporphyrins synthesized by oral microorganisms.[35] Porphyrins and metalloporphyrins typically have absorption maxima between 398 and 421 nm and emission maxima between 530 and 633 nm.[24] Because porphyrins are produced by bacterial metabolic activity, the red fluorescence intensity reflects the intensity and extent of the caries-affected tissues. Spectrographic studies have shown that carious dentine fluoresces more intensely in the red portion of the visible spectrum than healthy dentine.[25,26] Thus, laser fluorescence can be used to detect caries based on differences in fluorescence intensity between carious and healthy dental tissue in the near-infrared spectral region.[25] In FACE, the cavity is exposed to a violet-blue light with a bandwidth between 370 and 420 nm to excite orange-red fluorescence in carious dentine. Next, the samples are analysed through a 530-nm long pass filter to filter out the scattered excitation light and allow observation of orange-red fluorescence. The filter also reduces the intensity of green autofluorescence from healthy dentine. This allows selective removal of infected dentine and preservation of non-infected tissue. Previous studies have shown that FACE is more effective than CE in removing bacterially infected dentine.[17–19] Moreover, histological analysis has shown that this technology removed much more infected tissue when compared with methods based on CME and caries dye.[17] However, data on the minimal invasive potential (MIP) of FACE are scarce; only one study on the subject, which used silicone impression to measure cavity volume, has been published to date.[18]

Micro-computerized tomography (micro-CT) is a non-destructive technique that allows volumetric characterization of the degree of mineralization of hard tissues and assessment of their mineral density (MD).[27,28] Recent literature has shown that the effectiveness of caries removal techniques can be evaluated non-destructively using micro-CT by comparing the internal tooth structure before and after caries removal.[13,27–30] The density value of 1.11 g/cm^3 was defined as the threshold density between carious and sound dentine.[13,27] Neves *et al.* recently proposed a detailed protocol using micro-CT to quantitatively assess the caries removal efficacy (CRE) and the MIP of caries removal technologies. This non-destructive method is becoming increasingly popular in dental research and may become the gold standard for evaluation of dentine caries removal technologies.[13,27,29,31–33]

FACE has been reported to achieve good results in the removal of infected dentine compared with CE, CME and LIF.[17–20] However, no data are available about the MIP of FACE. The aim of this study was to compare the CRE and MIP parameter of FACE to those of CE, CME and LIF using micro-CT.

MATERIALS AND METHODS

Tooth selection

Molars and premolars with occlusal caries extracted for clinical reasons were collected from patients at the Oral Surgery Clinic of the West China College of Stomatology at Sichuan University. The molars and premolars were stored in 0.1% (w/v) thymol in the dark for up to eight weeks. The teeth were restoration free and showed no signs of tetracycline staining. The Ethics Committee of the West China College of Stomatology at Sichuan University approved the study. Each patient was informed of the objectives of the study and signed an informed consent form.

After ultrasonic cleaning, each specimen was radiographed. Teeth without dentine caries or teeth with a carious lesion <1 mm from the pulp chamber were excluded to avoid the risk of pulp exposure, leaving a sample size of 92. Radiographs were used to classify the lesion size for stratified randomization. The teeth were mounted in acrylic resin at the root (Sampl Kwik, Buehler, Lakebuff, IL, USA) for easy manipulation.

Baseline micro-CT scan

Micro-CT scans of the crown of each tooth were performed using a μCT 80 desktop micro-CT instrument (Scanco, Wangen-Brüttisellen, Switzerland). The same scanning parameters were applied to all teeth. The acquisition settings employed were 114 mA, 70 kV, 10 μm pixel size, and a rotation step of 0.7°. The resulting volume measurement was set as the baseline value.[36] A cut-off point corresponding to a MD value of 1.11 g/cm^3 HAp (as determined previously by correlating micro-CT grey values with hardness values of carious dentine[30]) was used as a cut-off point to segment each tooth into sound and carious volume in both the baseline and excavated caries section images.

Caries excavation

A single experienced dentist (XMZ) performed all of the caries removal procedures. Prior to the preparation of the cavity, all enamel overhangs from each lesion were removed in a minimally invasive procedure using a cylindrical diamond bur (Shofu Dental Equipment Co. Ltd., Japan) in a high-speed air turbine under water cooling until the underlying dentine lesion was exposed.

The 92 teeth that met the inclusion criteria were randomly divided into four groups according to the endpoint modality used (Table 1). Five teeth were excluded because the dentinal carious lesion was too small and seven other teeth were later excluded because of pulp exposure after caries excavation. The caries removal techniques tested are listed in Table 1.

In the CE group, the caries were removed using a tungsten carbide round bur (Shofu Dental Equipment Co. Ltd.) with a low-speed (approximately 1500 rpm) handpiece. This procedure was performed without water cooling.[13] Different sized burs were used depending on the size of the carious lesion. The caries removal endpoint was reached when a hard cavity floor was felt upon gentle pressure with a blunt dental explorer. The stained but hard dentine tissue was not removed in the procedure. The operating field was illuminated using a standard dental light unit.

The CME group was treated using a special Carisolv hand instrument (MediTeam, Goteborg, Sweden) according to the manufacturer's instructions. The cavity was covered with Carisolv gel (MediTeam) for 30 seconds. An excavator was then used to remove the carious tissue. This procedure was repeated until the caries removal endpoint based on the self-limiting capacity of the solution was reached.

Table 1. Caries excavation methods and respective caries removal endpoint used

Group	Caries excavation method	Caries removal endpoint	N
CE	Bur + blunt dental explorer	Hard cavity floor felt with a blunt explorer	18
CME	Carisolv + metal excavator	Self-limiting caries removal ability of the gel	20
LIF	Bur + DIAGNOdent	The mean value is lower than 15	20
FACE	Bur + red fluorescence marker	No red-orange fluorescence could be seen	22

In the LIF group, the bur was applied for caries removal and the DIAGNOdent was used to define the endpoint. The instrument was calibrated using a ceramic standard according to the manufacturer's instructions. The samples were dried briefly using compressed air. Tip A was applied and placed on the site to be measured. Measurements were repeated at several sites. Three consecutive measurements were performed and the average DIAGNOdent reading at the marked lesion area was used to determine whether to continue or stop excavating. The cut-off value corresponding to the measurement for healthy tissue was set at 15.[34]

In the FACE group, the cavity was excited using a 35 watt Xenon-discharge lamp and a blue band pass filter with peak transmission at 370 nm (Inspektor Research Systems Bv, Amsterdam, The Netherlands). Under these conditions, sound dental hard tissue fluoresces green and carious dental hard tissue fluoresces orange-red.[25] The operator inspected the cavity through a 530 nm yellow glass filter (OG530, Schott, Mainz, Germany) and removed orange-red fluorescing dentine, whereas green fluorescing areas were conserved. The room was darkened during the excavation procedure.[18]

Micro-CT analysis

Caries removal efficacy

The CRE was evaluated using two parameters: (1) the mean relative volume of residual caries to initial caries (RC/IC) and (2) the mean MD at the bottom of the cavity. The lower the RC/IC ratio, the more effective was the excavation of the caries. After recording the average MD, each caries excavated tooth was further classified as sound if the mean MD at the cavity floor was above the dentine caries cut-off point (1.11 g/cm3 HAp) or as carious if the average MD was lower than the cut-off point.[27]

Minimal invasiveness potential

The MIP of the different caries excavation techniques was evaluated by the relative cavity size (i.e. the PC/IC ratio).[13,18] A relative cavity size close to 1 indicates a perfect MIP, as the volume of the removed tissue (PC) corresponds exactly to the volume of the initial carious lesion (IC volume).[13] Figure 1 shows an example of the analysis performed based on the micro-CT data.

Statistical analysis

The CRE and MIP parameters measured for the four caries excavation techniques were compared by

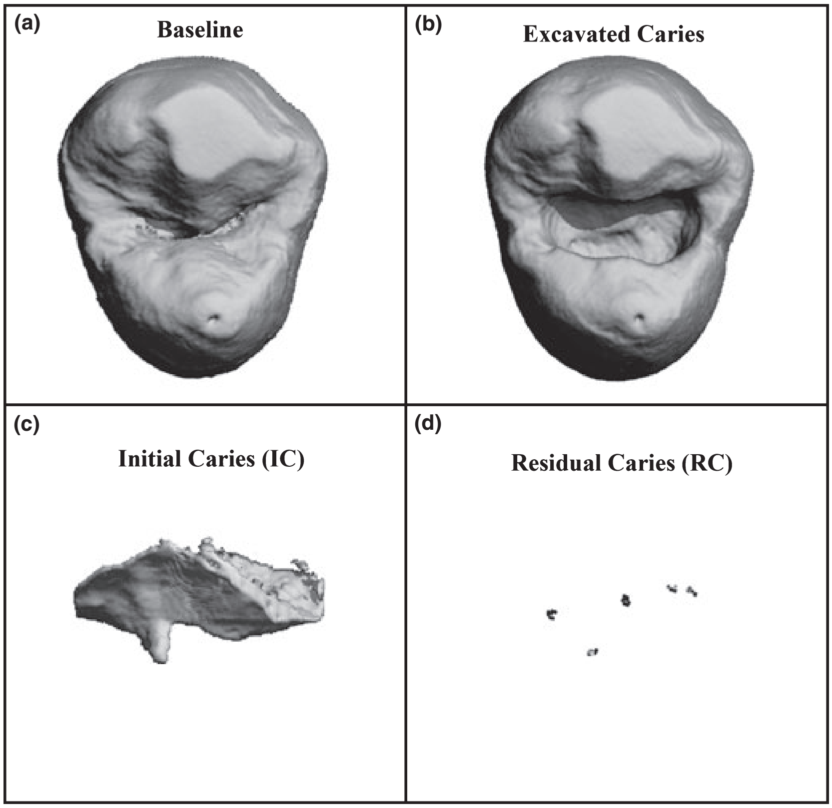

Fig. 1 3D volume renderings obtained from micro-CT cross-section slices of a tooth before and after caries excavation based on a previously defined cut-off point for dentine caries. (a) 3D volume of a tooth before caries removal (baseline); (b) 3D volume of the same tooth after caries removal (excavated caries); (c) 3D volume of initial caries (IC) after application of the cut-off point (1.11 g/cm^3 HAp) at baseline; (d) 3D volume of residual caries (RC) after application of the cut-off point (1.11 g/cm^3 HAp) to the excavated caries. The prepared cavity (PC) volume was obtained by subtracting the volume of the excavated tooth (b) from the volume of the tooth before the removal of the caries (a).

one-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) test. The difference in percentage of sound versus carious teeth for the four caries excavation techniques was statistically assessed using Fisher's exact test. The significance level was set at 0.05 for all analyses. The statistical analyses were carried out using the statistical software package SPSS 17.0 (SPSS, Chicago, IL, USA).

RESULTS

Table 2 shows the micro-CT results. One-way ANOVA results showed that the volume of IC did not differ significantly among the four groups. The highest RC volume was in the CME group (3.01), and this group was statistically different from the other three groups ($p < 0.05$). Among the CE, LIF and FACE groups, the difference in RC volume was not significant. The highest PC volume was in the LIF group (44.61), but the differences in this value among all four groups were not significant ($p > 0.05$). Tables 3 and 4 show the CRE and MIP results and the statistical differences for the comparisons among the different caries excavation techniques investigated.

The CE group has the second highest RC/IC parameter value (0.131); it was only lower than that of the CME (0.239) group. The difference between the CE and the CME groups was statistically significant ($p < 0.05$). The MIP value of the CE procedure was also the second highest (3.946); it was only lower than that of the LIF group (4.471). However, the difference between the CE and the LIF groups was not statistically significant. These results indicate that the CE procedure has some tendency towards over-excavation of the carious lesion.

Table 2. Micro-CT results

Group	RC(mm^3)	PC(mm^3)	IC(mm^3)	Mean MD(g/cm^3)	Sound dentine(%)
CE	1.50 ± 0.41	45.32 ± 8.67	11.49 ± 3.42	1.21	77.8
CME	3.01 ± 0.34	28.11 ± 7.49	12.61 ± 2.78	1.01	50.0
LIF	0.79 ± 0.19	44.61 ± 10.34	9.98 ± 1.81	1.32	80.0
FACE	1.26 ± 0.22	35.02 ± 11.01	10.95 ± 2.49	1.13	91.0

Table 3. Comparison results of CRE among each group

Group	(Sound dentine%)			
(RC/IC value)	CE (77.8)	CME (50.0)	LIF (80.0)	FACE (91.0)
CE (0.131 ± 0.110)				
CME (0.239 ± 0.120)	*			*
LIF (0.079 ± 0.076)		**		
FACE (0.115 ± 0.071)		*		

*Compared with other groups, ANOVA analysis revealed a significant statistical difference ($p < 0.05$).
**Compared with other groups, ANOVA analysis revealed a significant statistical difference ($p < 0.01$).

Table 4. Comparison results of MIP among each group

Group (PC/IC value)	CE	CME	LIF	FACE
CE (3.946 ± 2.530)				
CME (2.230 ± 1.741)	*			
LIF (4.471 ± 3.230)		**		
FACE (3.197 ± 1.581)				

*Compared with other groups, ANOVA analysis revealed a significant statistical difference ($p < 0.05$).
**Compared with other groups, ANOVA analysis revealed a significant statistical difference ($p < 0.01$).

The CME group had the highest RC/IC value (0.239) among all methods tested, and the differences between it and the other groups were statistically significant (CE group, $p < 0.05$; FACE group, $p < 0.05$; LIF group, $p < 0.01$). The mean MD ($1.01 g/cm^3$) at the cavity floor of this group was lower than the threshold MD ($1.11 g/cm^3$), and this was true only for this method. The MIP value for the CME group (2.23) was the lowest among the methods tested, and it differed significantly from the value for the CE group ($p < 0.05$) and the LIF group ($p < 0.01$). These data indicate that the CME technique results in excessively conservative caries removal, with a significant amount of carious tissue remaining compared with the other three methods.

The LIF group had the lowest RC/IC value (0.079), which was significantly lower than that of the CME group ($p < 0.01$), but the differences in this value among the LIF, CE and FACE groups were not statistically significant. The MIP value of the LIF group was the highest among the methods tested (4.471), and it was significantly higher than that of the CME group (2.23, $p < 0.01$). These results indicate that the LIF method has a tendency to cause over-excavation.

The FACE group had the second lowest RC/IC value (0.015), and it was not significantly different from that of the lowest group (LIF, 0.079). The mean MD value of the FACE group came closest to $1.11 g/cm^3$ ($1.13 g/cm^3$), and the caries-free ratio of the FACE group was the highest among the methods tested; only 2 of 22 teeth tested had an average MD lower than $1.11 g/cm^3$ at the cavity floor. These results show that the caries removal ability of this method is very consistent and effective. The MIP parameter of the FACE group (3.197) was higher than that of the CME group, but the difference was not statistically significant.

DISCUSSION

Our comparison of four caries removal techniques showed that FACE technology can leave a cleaner dentine floor than the CME method and preserve more sound dentine tissue than the LIF and CE methods.

The ideal caries excavation technique would be the one that selectively removes the irreversibly destroyed tissue but leaves the potentially remineralizable tissue at the cavity floor. Because a larger initial caries cavity means a larger cavity surface, comparison of RC volume only would not be valid for making comparisons among excavation methods. However, the relative volume of residual caries (i.e. RC/IC) should provide a fair comparison among techniques.

The CRE parameter can partly reflect the irreversibly destroyed tissue remaining after caries excavation, and the MIP parameter can partly reflect the tissue conservation condition. The CRE and MIP parameters are conflicting factors, but the selection of an effective caries excavation method should take both factors into consideration. This is hardly achievable clinically because even the currently available caries excavation techniques are not sufficiently specific to enable removal of only the irreversibly destroyed carious tissue. Indeed, some sound or at least potentially remineralizable tissue is frequently sacrificed to ensure that little bacteria-infected dentine remains.

In our study, both CRE and MIP were evaluated. The FACE technology had the best caries removal ability among the four methods tested. The orange-red fluorescence used as the caries removal marker is caused by porphyrins, which are by-products of the metabolism of oral microorganisms. Using porphyrins as a marker, the target is the bacterium. Our findings are consistent with those of Lennon *et al.*[20] who proposed FACE technology in 2002. The authors performed detailed studies and further developed this method in subsequent years. Various researchers have used histology to characterize the bacteria left after caries excavation using the FACE technology.[18] After application of the FACE method to excavate caries, teeth sections were evaluated for the presence of bacteria in the dentin tubules using light microscopy[19,35] or laser-scanning confocal microscopy.[18,20] Those studies confirmed that the FACE method removes more caries-affected tissue than CE or caries dye. Only one previous study compared the cavity size after caries excavation by FACE and other caries removal methods.[18] In that study, silicone impressions of the cavity made before and after caries excavation were compared. In the present study, micro-CT analysis not only enabled us to estimate the cavity volume more precisely than methods based on impressions, but it also enabled us to evaluate the MD at the cavity floor, which is related to demineralization of the tissue left after excavation.

The LIF group had the lowest volume of residual caries, followed closely by the FACE group, and the two groups did not differ significantly in RC/IC ratio. The differences in the latter parameter were statistically significant compared with the other two groups. A possible explanation for the similarity in results between the LIF and FACE technologies is that they both are based on a similar principle, as both detect bacterial metabolites. However, the LIF group had the highest MIP among the four methods tested, and the standard deviation of the MIP parameter was the highest. These findings support the previous report that DIAGNOdent is influenced by staining of the residual dentine.[13] The dark but hard dentine is usually over-excavated. Meanwhile, the percentage of sound dentine in the LIF group (80%) was lower than that in the FACE group (91%). This means that the FACE technology is much more reliable than the LIF technology. The sharp tip employed in the LIF method may be the reason that the DIAGNOdent reading is not stable as it can only focus on very tiny areas, and thus each measurement may differ greatly from other readings. This makes the LIF method very sensitive but also imprecise.[29]

In our study, the CME group had the best MIP and the worst CRE. These CRE and MIP values are similar to the results of a previously published study that compared nine kinds of dentine caries removal methods.[13] However, that study did not include the FACE method. Among the nine methods studied, CME was classified as the best when both MIP and CRE were taken into consideration.[13] In another study, Lennon *et al.* compared FACE with CME and found that FACE left less bacterially infected dentine after excavation than CME.[35] They concluded that FACE is more effective at removing infected dentine without significantly increasing cavity size when compared to CE. The relative cavity volume (MIP) of FACE in their study was 3, which is similar to our result of 3.19. Our findings are also consistent with Lennon *et al.* who suggested that the CE method has a tendency to over-excavate the carious lesion. In our study, after excavation the smallest cavities relative to the original lesion size were obtained in the CME group, but the FACE group had the second smallest average cavity size. Moreover, the differences between these groups were not statistically significant. Overall, the data indicate a greater accuracy of the FACE method when compared with the other methods as the bacteria were more effectively removed without increasing the cavity size.

FACE technology does have some limitations. Because FACE technology is based on light, fully opening the cavity is the first step in the process. This method may remove unsupported but sound enamel. The size of the light source and camera also restrict the range of application of this technique. For example, it is more sensitive for treating the cavity floor than the cavity walls. This issue could be improved by using a smaller light source and camera. In addition, the current study had several limitations. First, extracted and stored teeth were used in this study, as waiting for fresh extracted teeth for the experiment was not practical and would have been very time consuming. The storage times for each tooth used in our study were not the same, and thymol, which was used as the preservative, has antimicrobial properties. Because FACE is based on bacteria, the phenomenon of red fluorescence needs to be compared between teeth *in vivo* and extracted teeth, especially those immersed into thymol. Second, FACE techonology only works on surfaces whose images can be clearly captured, such as the cavity floor, and the judgement is also focused on the cavity floor. Thus, the conclusion that FACE technology is better than the other methods tested should be confirmed in further studies.

CONCLUSIONS

Based on our results we draw the following conclusions. Both CRE and MIP parameters of FACE technology were the second most acceptable and there was no statistical difference compared with the most acceptable. Taking both CRE and MIP into consideration, the FACE method is effective in removing heavily infected dentine without significantly increasing cavity size compared to the other three methods *in vitro*.

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