

# Fluorescence-Aided Caries Excavation (FACE) Compared to Conventional Method

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## Clinical Relevance

A new caries excavation method that uses a fluorescence diagnostic procedure during excavation allows the operator to identify and remove bacterially-infected dentin more successfully than with the conventional method that uses visual tactile criteria for identification of caries.

## SUMMARY

A recent study showed that orange-red fluorescence in carious dentin could be used to detect residual caries (Lennon & others, 2002). This study compared the ability of a new fluorescence-aided caries excavation technique (FACE) with the conventional method. Forty extracted teeth with occlusal dentin caries were selected. The teeth were bisected longitudinally through the center of the lesion. Lesion depth and width were measured and the teeth were divided into two groups of 20, each with the same average lesion size. The tooth halves were reassembled and fixed by embedding the roots in acrylic resin. Access cavities were prepared using a high-speed handpiece and diamond fissure bur. In the FACE group, violet light (370–420 nm) was fed into the fiber optics of a slow-speed hand-

piece, so that it illuminated the operating field. The cavity was observed through a 530-nm high-pass filter and orange-red fluorescing areas were removed. In the conventional group, a sharp probe was used to detect soft dentin, which was removed. One-half of each tooth was stained for bacteria using Ethidium Bromide and examined using Confocal Laser Scanning Microscopy (CLSM). Bacteria were present in significantly ( $p=0.037$ ) fewer FACE samples (3) compared to conventional samples (9). It can be concluded that the new method is more effective than conventional caries excavation.

## INTRODUCTION

Most of the dental restorations placed in Scandinavia, the UK and the USA during the last 20 years were replacements rather than initial restorations (Deligeorgi, Mjör & Wilson, 2001). If infected dentin is not completely removed before placing a restoration, caries can recur. Most commonly, dentists decide whether dentin should be excavated or not based on the color and hardness of the tissue. This decision is often difficult clinically and recurrent caries is still one of the major reasons for restoration replacement (Dahl & Eriksen, 1978; Pink, Minden & Simmonds, 1994).

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Caries detector dyes were introduced in the 1970s to help identify infected dentin (Sato & Fusayama, 1976). Kidd, Joyston-Bechal and Beighton (1993) have shown that there is no difference in the level of infection of dye-stained and dye-unstained sites at the Dentin Enamel Junction (DEJ) and concluded that the use of a caries detector dye on hard and stain-free dentin will result in unnecessary tissue removal. Since these dyes also stain normal circumpulpal dentin, their use may result in unnecessary removal of healthy tissue (McComb, 2000). Chemomechanical systems recently introduced for caries removal have also been investigated. However, their usefulness at the Dentin Enamel Junction (DEJ) appears to be limited (Cederlund, Lindskog & Blomlöf, 1999a) and there are concerns that they may damage the collagenous component of dentin which is needed for adhesive restorations (Cederlund, Lindskog & Blomlöf, 1999b). Therefore, an accurate and reliable method for residual caries detection is still needed.

Because bacteria in dentin are not visible to the observer, methods for detection of residual caries have focused until now on identifying tissue that has already been damaged by the caries process, for example, demineralized dentin (caries detector dyes) or dentin where the collagen has been denatured (chemomechanical methods [Carisolv]). However, several oral microorganisms are known to produce fluorescing molecules or "fluorophores" that emit in the yellow to red area of the visible spectrum under certain excitation wavelengths (König & Schneckenburger, 1994).

A recent *in vitro* study showed that exciting carious dentin with violet blue light caused visible orange-red fluorescence that could be used successfully to identify residual caries (Lennon & others, 2002).

This study evaluated a new caries excavation technique that is based on the previously described fluorescence diagnostic procedure.

## METHODS AND MATERIALS

### Specimen Preparation

Extracted human premolars and permanent molars with occlusal caries were collected and stored in 0.01% thymol solution. All teeth were sectioned longitudinally under continuous water cooling (Ultraslice 2000, Ultratec, Santa Ana, CA, USA) through the center of the lesion in a mesiodistal direction. After examining the tooth halves using stereomicroscopy, 40 samples with caries at least 1 mm into dentin and 1 mm clear of the pulp were chosen. The selected teeth had a single occlusal lesion and were free of any restorations.

Lesion depth and width were measured and the teeth were distributed into two groups of 20 teeth, each group having the same average

and total lesion size. Tooth halves were reassembled and fixed by embedding the roots in acrylic resin (Sampliquik, Buhler, USA) to the level of the DEJ.

### Excavation

Caries excavation was carried out by one operator for both groups. No magnification was used. Samples were removed from the storage solution for excavation. They were then replaced in storage solution immediately after excavation.

### FACE Group

Access cavities were prepared using a 557-diamond bur in a high-speed handpiece (Star Dental, Lancaster, PA, USA) under continuous water-cooling. Violet light (370–420 nm) was generated using a 35-watt Xenon discharge lamp and a blue band pass filter with peak transmission at 370 nm (taken from QLF system, Inspektor Research Systems BV, Amsterdam, The Netherlands). This light was fed into the fiberoptics of a slow-speed handpiece (Star Dental) so that it illuminated the operating field during excavation (Figure 1). The operator observed the cavity through a 530-nm yellow glass filter (OG530, Schott, Mainz, Germany) in a darkened room. Areas exhibiting orange-red fluorescence were selectively removed using stainless steel round burs sizes 4 and 6.

### Conventional Excavation Group

Access cavities were prepared using a 557-diamond bur in a high-speed handpiece (Star Dental) under continuous water-cooling. During excavation samples were illuminated using a standard dental unit light. Brown and yellow stained dentin and/or softened dentin detected using a sharp explorer (EX85, Hu-Friedy Inc, Chicago, IL, USA) were removed from the DEJ. Soft dentin was removed from the rest of the cavity. Caries

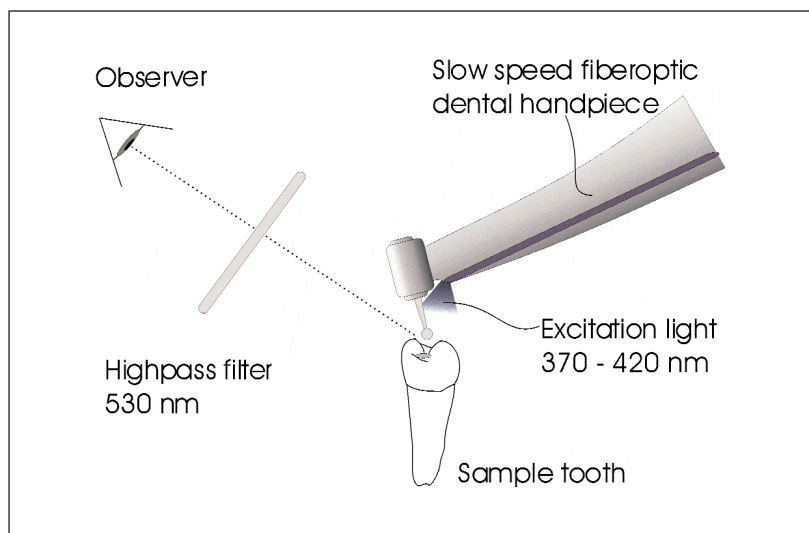


Figure 1. FACE excavation method.

was removed using stainless steel round burs sizes 4 and 6 in a slow-speed handpiece (Star Dental).

### Histology

The acrylic base and roots of each sample were removed (Ultraslice 2000, Ultratec, Santa Ana, CA, USA) and the teeth were disassembled into two halves. One half of each sample was used for histology. Samples were fixed in 70% ethyl alcohol, then rinsed extensively in phosphate buffered saline, pH 7.2 (PBS). Samples were placed in a 1:500 concentration of 10 mg/ml ethidium bromide (Molecular Probes, Eugene, OR, USA), vortexed and incubated at 37°C for 20 minutes. Samples were then rinsed extensively in PBS.

The tooth halves were analyzed for the presence of fluorescent stain using a confocal laser-scanning microscope (Odessey, Noran Instruments, Middleton, WI, USA). A 488 nm argon ion laser was used for excitation. A 515-nm barrier filter, a 15 µm confocal detection slit and a 60X oil immersion objective were used for detection. Scans were made at a depth of approximately 10 µm below the cut surface and along the cavity outline. The sample edge was excluded to avoid false positives due to surface contaminants.

Samples were scored positive for residual caries when bacteria were identified. Samples were scored negative for residual caries when no bacteria were identified.

### Statistical Analyses

The difference between the two groups was statistically analyzed using the Pearson Chi squared test. The level of significance was set at  $p < 0.05$ .

Data were analyzed using SPSS software version 10.1 for windows.

## RESULTS

A cross-tabulation of the results is presented in Table 1. No pulp exposures occurred. All samples had sufficient dentin remaining after excavation to allow staining and analysis using CLSM. In the conventional group, residual caries was detected in nine samples (Figure 3). The remaining 11 samples were caries free. In the FACE group, residual caries was detected in three samples (Figure 2), while 17 samples were caries free. Residual caries was detected in significantly fewer FACE samples than conventionally excavated samples ( $p = 0.037$ ).

## DISCUSSION

This paper describes, for the first time, the use of a new dental handpiece that combines a fluorescence diagnostic procedure with caries removal. In a previous study, it was shown that visible orange-red fluorescence in carious dentin could be used to detect residual

caries (Lennon & others, 2002). In the earlier study, however, flat samples were used and caries was removed by grinding. In contrast, both the samples and the excavation procedure used in the current study

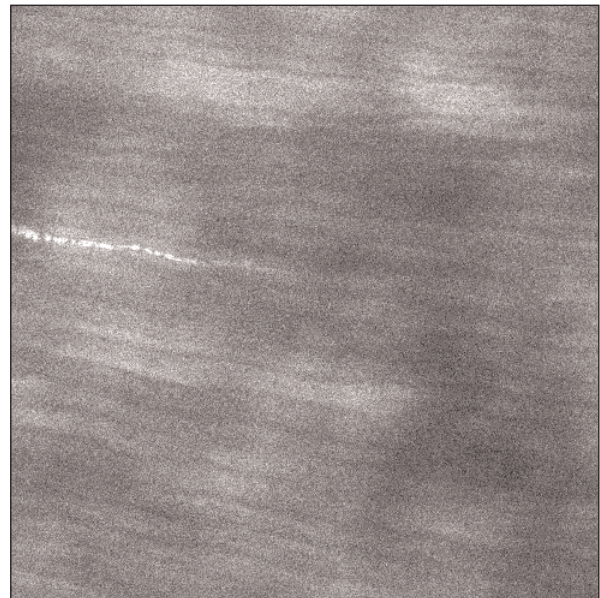


Figure 2. Confocal micrograph of sample with residual caries following FACE excavation (1720x).

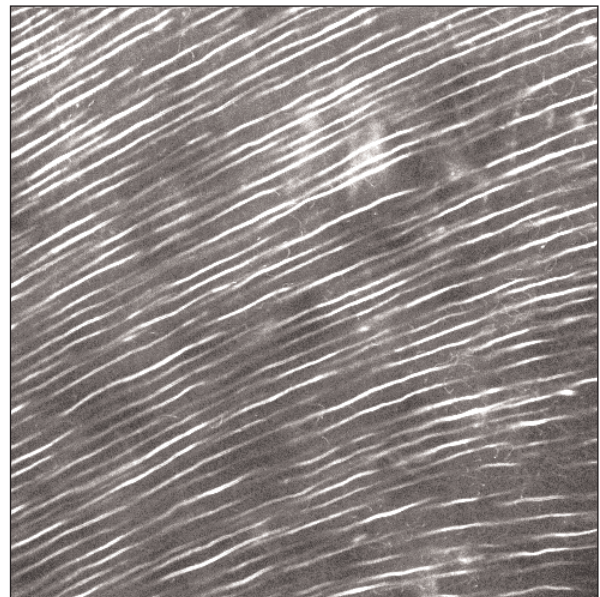


Figure 3. Confocal micrograph of sample with residual caries following conventional excavation (1720x).

Group	Caries Free	Residual Caries	Total
FACE	17	3	20
Conventional excavation	11	9	20
Total	28	12	40



were three-dimensional and therefore similar to the clinical situation.

Porphyrins and metalloporphyrins produced by some oral microorganisms as metabolic byproducts are thought to be responsible for orange-red fluorescence in carious dental tissues (König, Flemming & Hibst, 1998). These fluorophores typically have absorption maxima between 398 and 421 nm and emission maxima between 530 and 633 nm (König & Schneckenburger, 1994). Because emission occurs in the visible portion of the electromagnetic spectrum, it can be detected by visual inspection using the appropriate high-pass filters (Lennon & others, 2002).

In this current study, teeth with caries extending very close to the pulp were excluded to avoid the possibility of pulp exposure and because samples should still have sufficient dentin after caries removal to allow staining and CLSM examination of the dentin tubules.

It is recommended that infected dentin be completely removed before a restoration is placed (Weerheijm & others, 1999). Demineralized but not infected dentin is thought to be remineralizable and should be conserved rather than removed (Fusayama & Kurosaki, 1972). Therefore, laboratory techniques to evaluate the success of excavation techniques should specifically detect infected dentin remaining after excavation (residual caries) rather than demineralization.

Confocal microscopy has been used in conjunction with immunofluorescent labels to identify bacteria in caries lesions in the past (Gonzalez-Cabezas & others, 1999). The disadvantage of using a specific antibody label for detection of residual caries is that only one bacterial species can be labeled. The microflora of dentin caries is complex (Pekovic & others, 1987) and, therefore, it is preferable to use a technique that reveals the presence of bacteria regardless of species. Ethidium bromide is a fluorescent nucleic acid stain that has been used extensively for identification of bacteria in plaque regardless of species (Netuschil, 1983). Because the odontoblast nucleus is situated at the border of the pulp chamber and not within dentin itself, a nucleic acid stain can be used to label bacterial nucleic material within the dentin tubules.

Although the new method was more successful in removing bacterially-infected dentin than conventional excavation, bacteria were still detected in three samples after FACE excavation. The main difference between CLSM and FACE is that CLSM specifically identifies bacteria, whereas, FACE detects fluorescence produced by bacterial by-products. Another difference is the specificity of the methods. The gold standard (CLSM) is capable of identifying a single bacterium, whereas, the FACE method relies on the abilities of the human eye and may not detect very small amounts of fluorescence. The numbers of bacteria present in the

positive FACE samples appeared to be much less than that in the positive conventional samples. However, bacteria present in the samples were not quantified and this would be an interesting question for future studies.

The gold standard used in this study tested the ability of the respective excavation methods to remove bacteria-infected dentin. An accurate excavation technique should, however, not only successfully remove infected tissue but also conserve sound tissue, and this aspect should also be addressed in the future.

## CONCLUSIONS

Within the limitations of this *in vitro* investigation, it can be concluded that excavation using FACE results in significantly fewer cases of residual caries than conventional excavation.

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