Dentine Caries: Take It or Leave It?

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Abstract: In modern dentistry the primary aim when excavating carious dentine is to eradicate only the highly infected, irreversibly demineralized and denatured biomass in order to allow effective restoration of the cavity, restoration of the surface anatomy of the tooth and to prevent disease progression. However, the boundary between this superficial zone of dentine requiring excavation and the deeper, affected but repairable tissue is not always obvious either in the clinic or in the research laboratory. The inherent subjectivity in detecting this excavation boundary can result in clinically significant differences in the quality and quantity of dentine removed by different operators and makes the *in vitro* comparison of newer excavation techniques more difficult. This article discusses the rationale behind carious dentine excavation and the criteria available to the dentist, both clinical and laboratory, to help identify the dentine requiring removal.

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Clinical Relevance: Current trends in modern dentistry involve the maximum conservation of tooth structure during operative treatment of carious dentine. This article focuses attention on the rationale behind its excavation and the alternative criteria available to the practitioner to distinguish the dentine requiring removal.

When the clinical decision to intervene operatively in the treatment of a carious lesion has been made and the overlying carious and unsupported enamel has been removed, the dentist is often faced with what, from the occlusal aspect, appears to be an unstructured mass of brown and softened tissue. However, if the same lesion is longitudinally hemisected in the laboratory and the flat cut surface visually examined, a more complex structure is revealed (Figure 1). Colour gradations within the dentine are clearly evident, ranging from dark brown subjacent to the enamel–dentine junction (EDJ) to a more glassy, opaque translucent zone at the lateral borders and advancing front of the lesion. The relative hardness follows a similar pattern, ranging from very soft at the EDJ to normal values in the dentine between the advancing front of the lesion and the pulp (Figure 2). How does this appearance relate to the histological structure of the dentine lesion and what the dentist can and should remove during operative treatment?

Fusayama and co-workers have shown, from the results of a series of histological experiments, that the coronal dentine lesion can be divided into two structurally distinct zones.[1] The outer, superficial zone has suffered the ravages of the carious attack for the longest period and ultimately consists of irreversibly acid-demineralized dentine. The collagen in this zone has been exposed to excessive proteolytic degradation, which prevents any reconstitution of the molecular cross-links (Figure 2, zone 1). The inner, deeper zone of the lesion comprises dentine that has been reversibly attacked by the carious process and thus still retains the potential to repair under suitable conditions. Here, even though the collagen has undergone proteolysis, the damage to the molecular cross-links is not as severe. It is thought that this zone should be retained at the cavity floor to allow repair/remineralization to occur after placement of a suitable restoration (Figure 2, zones 2, 3 and 4). Related to these structural zones are the bacterial penetration front and the acid penetration front. Fusayama discovered a possible coincidence between the boundary of the structurally damaged superficial zone and the highly infected biomass of dentine found within the whole lesion.[2] It was argued that the dentine deeper in the lesion was only minimally infected and thus could be retained as long as the bacteria remained inactive. It is interesting to note that these

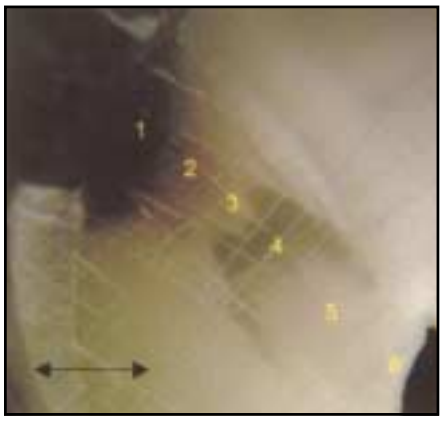

Figure 1. *Reflected light photomicrograph of a cavitated, approximal lesion sectioned longitudinally, showing colour gradations from the heart of the lesion (underlying the EDJ) to the advancing lesion front. These zones appear to be impossible to distinguish objectively and difficult even to distinguish subjectively, in this section (bar = 1 mm).*

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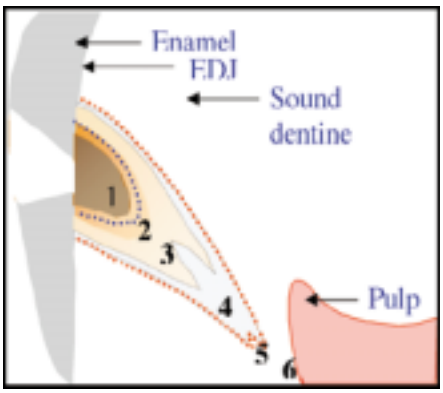

Figure 2. *Histological zones of carious dentine in an active, approximal lesion.* ***1*** *Outer layer of irreversibly denatured and demineralized, infected dentine.* ***2–4*** *Combine to form the inner layer of reversibly demineralized, affected dentine.* ***3*** *Transparent layer of carious dentine.* ***4*** *Sub-transparent layer of carious dentine (3 and 4 are layers known as translucent dentine or 'sclerotic dentine').* ***5*** *Hardest layer of sound dentine.* ***6*** *Softer layer of sound dentine adjacent to pulp. Acid penetration front. Bacterial penetration front.*

experiments were accomplished using conventional bacterial culturing techniques; it has now been shown that these methods are capable of detecting less than 50% of the total bacterial population in oral tissues.[3] Therefore, it seems prudent to re-examine this microbiological distribution.

WHAT SHOULD WE EXCAVATE?

In a patient where preventive treatment alone has not halted the carious process, an important consideration during operative treatment is the quantity and the quality of the dentine being removed. The numbers of bacteria (i.e. the infectivity) and the depth of penetration into the dentine may be important factors to consider as the purpose of excavation is to remove the soft, irreversibly damaged and highly infected tissue before placing the restorative material. It is then hoped that the completed restoration will prevent further progress and spread of the lesion. There are four possible ways by which the carious process in dentine can be arrested with operative intervention:

- by restoring the integrity of the tooth surface, thus allowing adequate plaque control to be re-established;
- by physically removing the highly infected tissue itself;
- by restricting the nutrient supply to the remaining bacteria in the dentine or
- by using bacteriostatic restorative materials (e.g. glass ionomer cements, zinc oxide/eugenol or calcium hydroxide-containing compounds) to inactivate or kill the remaining microbes.

It is important to question the relevance of each of the above in arresting the carious process. For instance, how important is it to actually remove *all* the infected tissue and can this reasonably be achieved? Early experiments showed that bacteria can remain viable beneath restorations for over a year[4] (admittedly, this study was not performed using any form of therapeutic lining material at the base of the prepared cavity). A later study corroborated these findings: quantities of lactobacilli and Gram-positive cocci were found, but again under non-antiseptic restorations.[5] If deep lesions close to the pulp are treated with an antiseptic lining material (e.g. calcium hydroxide) or cariostatic restorative materials (e.g. glass ionomer cements) then residual bacteria retained beneath the restoration will be very unlikely to cause further problems.[6–11] Some researchers have used demineralized histological sections stained for bacteria to assess their persistence after excavation. Shovelton[7] found that approximately 40% of the examined teeth 'had some infected tubules on the pulpal floor' and Crone arrived at similar conclusions on the basis of histological examination of 113 extracted teeth after rigorous excavation of deep carious lesions.[8] Shovelton again concluded, in agreement with Crone, that in many cavities prepared by classical techniques some bacteria still persist.[8,9] None of these studies assessed the actual activity of the remaining bacteria and their ability to cause disease progression. Indeed, it has been observed by Bjørndal *et al.* that, even though an increase in clinical hardness was obtained after caries excavation, bacterial recovery was still often seen in discoloured dentine.[10]

Collectively, these studies show that it is probably not possible to remove all the infected tissue – but they also question the ultimate necessity of doing this. This challenges current concepts of how carious dentine should be managed. If bacteria are retained within the cavity, what is likely to happen to them over time? Will they survive? If they do, they will require nutrients, the sources of which might include:

- the oral environment/plaque (via marginal leakage of restorations);
- the pulp (via patent tubules/channels/porosities and odontoblastic processes);
- other bacteria (via complex bacterial ecosystems in deep dentine lesions);
- the degraded tissue in which they reside.

It has been argued that, if infected dentine is sealed with a well-adapted restoration, the numbers of remaining bacteria reduce over time[10] (these studies again used standard culturing methods to identify the presence of bacteria within the cavities). Bjørndal *et al.* found that the dentine, after initial excavation of the carious biomass followed by restoration including a calcium hydroxide lining, turned a darker brown colour as well as becoming harder and more leathery in consistency.[10] These findings were interpreted as a sign of arresting caries, in agreement with other research studies.[12,13] Even though the bacterial culture method tested the viability of the bacteria (i.e. their continued ability to replicate under controlled conditions), it was still difficult to test accurately the actual activity of the bacteria *in situ* with this method of analysis. Mertz-Fairhurst *et al.* assessed the progress of caries clinically in restorations where carious dentine was left *in situ* but sealed from the oral environment.[11] These restorations were followed over a 10-year period; results showed that in well-sealed adhesive restorations no obvious clinical or radiographic signs of caries progression could be detected. This, indeed, implies cessation of the carious process. On the other hand, Weerheijm *et al.* examined

the presence of bacteria sealed beneath amalgam and resin-modified GIC occlusal restorations over a 2-year period.[14] Even though they found that numbers of bacterial colony-forming units (*Streptococcus mutans* and lactobacilli*)* had reduced, they suggested that judicious excavation was sensible in order to prevent the chance of re-activation of the remaining bacterial population.

The principle of excavating the central carious biomass is indirectly applied in the Atraumatic Restorative Treatment (ART) technique, in which simple hand excavation followed by restoration with glass ionomer cement is advocated to prevent further progress of the disease.[15] The likelihood of bacteria obtaining sufficient substrate from the pulp is minimal owing to the lack of patency of the tubules (especially in the translucent zone – see Figures 1 and 2)[16] and the presence of tertiary/reparative dentine, with its distinct lack, and disorganization, of tubular structure. The problem with these therapies in the long term, however, is the risk of failure of the marginal seal and the persistent lack of information regarding the ultimate viability and activity status of the remaining bacteria. Might a new supply of nutrients re-awaken dormant but viable bacteria, which could then re-activate the carious process in the depths of a cavity? The answer to this important question is not known at present so it would seem sensible to continue advocating the removal of the highly infected outer zone of dentine. This will ensure that the vast majority of the bacterial population will have been eradicated and that the action of the remaining few will be nullified by sealing them off with a well-sealed bacteriostatic restoration.

HOW CAN THE EXCAVATION BOUNDARY BE IDENTIFIED?

Having discussed the rationale behind carious dentine requiring clinical removal, it now seems logical to discuss how a dentist can identify this tissue; or, to put it another way, how does the dentist know at what level to stop excavating? There are several parameters available to help highlight the excavation boundary (see Table 1) but an inherent problem with these clinical criteria is the inability to standardize them between different lesions and different operators.

Criterion	Comments
Hardness	Clinical: subjective; Laboratory: objective
Colour	Clinical or laboratory: subjective
Caries dyes	Clinical or laboratory: subjective
Bacteria	Laboratory: objective
Autofluorescence	Laboratory: objective

Table 1. *Criteria for identifying carious dentine requiring removal.*

Colour

It is not clear at present why carious dentine has a brown coloration. Studies have investigated the possibility of extrinsic stain being incorporated into the carious tissue perhaps due to its increased porosity, either from the oral cavity or the pulp.[17] However, more recently emphasis has been placed on a biochemical reaction occurring between carbohydrates and proteins in an acidic environment, not unlike that found in the depths of a carious dentine lesion. This reaction is known as the *Maillard reaction*,[18] but convincing evidence has yet to be found of it actually occurring *in vivo*.

Colour perception is very subjective, and is affected by many factors including the ambient lighting, the state of hydration of the tooth surface and the natural history of the lesion. The degree of dentine lesion pigmentation is not constant (see Figure 1), with variations occurring due to the age of the lesion and the site within the lesion as well as its state of activity (i.e. whether it is arrested or rapidly progressing). There is no clear correlation between the colour of a lesion and its state of demineralization or infectivity,[19, 20] a fact that is highlighted in the schematic representation of the cavitated lesion in Figure 2, which summarizes the poor link between the colour, bacterial penetration and the acid front.

Hardness

This is a criterion frequently used by dentists to distinguish the dentine they wish to remove during excavation. The hardness varies through the depth of a lesion, obviously being markedly softened subjacent to the EDJ, in the heart of the lesion. As one progresses towards the advancing front of the lesion, towards the pulp, the hardness gradually increases, reaching a peak in the sound dentine underlying the 'sclerotic' zone.[20,21] The sclerotic zone has been described as being made up of two layers, transparent and sub-transparent.[22] Ironically, the hardest dentine is actually found just below these and therefore the name 'sclerotic' is a misnomer. It would be more accurate, perhaps, to call the glassy, opaque zone at the advancing front the *translucent zone*.

Investigations have indicated a possible clinical correlation between the markedly softened dentine and its level of infectivity. Kidd *et al.* have argued that, as long as the softened, wet dentine in the heart of the lesion has been removed, this process will clear the cavity of the highly infected dentine, irrespective of its final colour.[19] There are two interesting points worth further discussion, however: first, hardness is relative and its assessment will therefore, by definition, vary between operators and second, the conventional methods used to analyse the bacterial content are potentially able to detect less than 50% of the total bacterial population.[3] So do we really know how many bacteria will be retained at the cavity floor and what species they are? Are these retained bacteria clinically relevant to the progression of the disease in dentine?

Caries Detector Dyes

In an attempt to eliminate the subjectivity in finding the excavation boundary, Fusayama and Terachima in 1972 developed a dye whose active ingredient was propylene glycol combined with a

visible dye stain.[23] This was said to bind to collagen cross-links and thus enabled discrimination between the two structural zones in carious dentine, reportedly staining only the outer, irreversibly damaged zone. This sounded like a promising solution to the problem, but unfortunately use of the dye can lead to significant clinical cavity over-preparation due to diffusion and porosity effects within the dentine.[20,24,25]

Bacterial Analysis

Many microbiological studies have attempted to find out which pathogens or combinations of pathogens, many initially residing in the surface plaque biofilm, are responsible for dental caries.[26] Even though certain species have been consistently found in such lesions (including *Streptococcus mutans*, lactobacilli and actinomyces) their interactions have yet to be ascertained, partly because caries progresses in a very complex ecosystem. Past studies used culturing methods to detect and quantify the type and numbers of bacteria present within lesions. However, historically, the most significant limitations in the field of oral microbial ecology have been those imposed by these cultivation-dependent techniques. There is now a belief amongst oral microbiologists that the cultivable fraction of bacteria is generally less than 50% of the total number present within sites of oral infection[3] and so the previous conclusions reached concerning the diversity, abundance and spatial distribution of micro-organisms from carious lesions based on these cultivation techniques may be an over-simplification of the true microbial status.

The development of techniques that avoid reliance upon cultivation of bacterial cells has revolutionized the understanding of microbial distribution. One such method is the use of phylogenetic signature nucleic acid targeted probes.[27,28] This technique has recently been used in the assessment of the microbial ecology within a carious coronal dentine lesion.[20,29] The most widely used nucleic acid target for the detection of bacterial cells in the environment is the 16S ribosomal RNA (rRNA) molecule. The general approach for studies using oligonucleotide probes has been to target discrete regions of the rRNA molecule for hybridization to phylogenetic group or species-specific oligonucleotide probes. The rRNAs of different organisms vary in relative sequence conservation, so that targeting regions of greater or lesser conservation allows for the design of probes with differing phylogenetic specificity. Some regions of rRNAs have remained essentially unchanged in all sequenced species; these can be used as targets for universal probes. DNA probes complementary to specific base sequences residing on the rRNA can be manufactured and tagged with a suitable fluorescent dye, enabling detection using the appropriate filter configurations with a fluorescence microscope.

Results from initial experiments have shown this technique to be very effective in labelling the bacteria directly within samples of dentine excavated from varying levels through a lesion[20,29] (Figure 3). Preliminary findings indicate that bacterial numbers in carious dentine fall away considerably at the advancing front of the lesion, but are still present to a greater degree than has been assumed.

One major advantage of this molecular technique is the fact that it might be possible to assess the activity state of the bacteria, because the genetic material that is being labelled (the rRNA) is manufactured in greater quantities when the cell is active. This, in turn, leads to the generation of more binding sites for the DNA probe and will manifest in the intensity of the final fluorescent signal detected. This use of molecular analysis is still in its infancy but as the methodology is refined it will no doubt help to shed more light on this important aspect of disease progression.

What is really thought-provoking is the ultimate success of most of the current restorative techniques, despite leaving so many bacteria beyond the excavation front. Logic demands that we should question their relevance in disease progression. Could we be basing our current concepts of caries removal on a faulty premise?

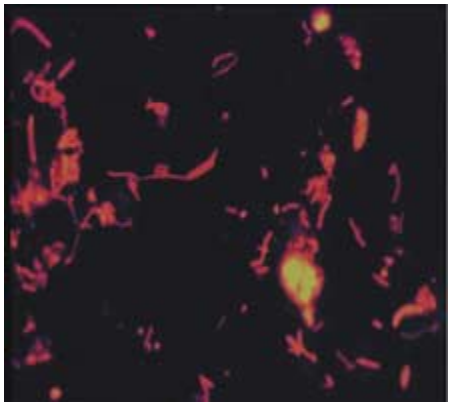

Figure 3. *Photomicrograph (100x/1.4 oil immersion objective) of bacteria labelled with rhodamine-tagged, universal DNA probe (EUB338). The sample of dentine was taken from the advancing front of the lesion and indicates the presence of rods, cocci and filamentous morphologies (fieldwidth = 100 microns).*

Autofluorescence

In simple terms, fluorescence occurs when a sample absorbs incident light of one wavelength, alters it and then re-emits it as light of a longer wavelength. It has been known for a number of years that carious dentine exhibits its own innate fluorescence, known as *autofluorescence* (AF).[30,31] Studies have been performed using confocal laser-scanning microscopy to generate and detect this signal in the laboratory using 488 nm blue light excitation and >515 nm yellow–green light emission.[20,32] Having minimized the errors induced by light scattering and sampling artefact, a series of investigations involving the AF signal and colour, hardness (both clinical and laboratory), infectivity and state of demineralization of natural carious lesions have, by a process of elimination, deduced that the AF signal is being generated by a chromophore residing in the altered carious dentine matrix, probably created by an interaction between bacteria and the dentine matrix within the heart of the lesion (Figure 4).[20,21,29,32]

Autofluorescence has also been used to help to identify the carious dentine that would be removed by hand excavation in laboratory experiments and compare this with the dentine that would be removed by alternative methods of caries excavation.[20,33] The AF signal detection offers

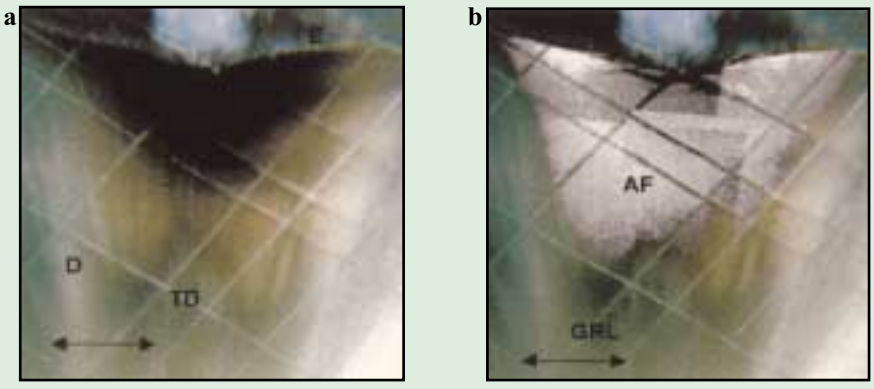

Figure 4. (a) *Reflected light photomicrograph of an occlusal lesion (E: enamel; D: dentine; TD: translucent dentine; bar = 1 mm).* ***(b)*** *Autofluorescent signature (AF) superimposed on the original image using the surface grid reference lines (GRL). Note how the signal emanates from the carious dentine only, within the pigmented lesion boundary, superficial to the translucent zone (TD).*

objectivity in the laboratory assessment of the position of the excavation boundary in histological sections. It cannot yet, however, resolve the problem of how much of the infected dentine should be removed.

SUMMARY

The rationale behind carious dentine excavation and the criteria used by dentists to guide tissue removal are by no means clear cut. The important features of operative dentistry include removal of the irreversibly damaged and denatured dentine, which is also highly contaminated with bacteria. However, studies contradict each other and the clinical operative guidelines used by dentists are inherently subjective in nature. All of this results in imprecision, the amounts and qualities of dentine removed during cavity preparation depending on the operator. From the evidence discussed, the relevance of this may be limited to the compromised bonding ability of current restorative materials and thus the overall sealability of the restoration.

Further investigation is required into the role of the bacteria within the dentine in disease progression and the search for clinically objective markers to aid consistent carious dentine removal between lesions and operators. It is vital, as practising dentists, to keep questioning what we do and why we do it. This is what ultimately sets the good clinicians apart from the rest.

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