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Fluoride-containing materials and the prevention of demineralization during orthodontic treatment – which research method should we now use?

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Abstract

With time cariologists have changed their understanding of the effects of fluoride on the caries process. These findings have been discovered predominantly through work carried out in laboratories. Whereas cariologists carry out laboratory studies to explain the mechanisms of action of fluoride and material scientists to test the properties and biocompatibility of materials, as orthodontists we are more interested in determining how useful a product is in clinical practice. In this article I will outline the advantages and disadvantages of laboratory methods designed to determine whether a fluoride-containing material is effective in reducing demineralization during orthodontic treatment. I will argue that the only way to test a material is to undertake a randomized clinical trial, with a parallel group design, over the full length of orthodontic treatment.

Introduction

The effectiveness of fluoride in reducing the incidence of dental caries has been known for many year(1), but with time cariologists have changed their understanding of how fluoride works. There is now general agreement that the main mechanism of action is not due to a systemic effect; by changing the enamel structure to form less soluble crystals as was originally thought, but due to a local effect; by changing the balance of the process toward remineralization(2). This occurs at fluoride levels as low as 0.01ppm(3). The critical pH for hydroxyapatite (the pH at which it starts to dissolve and hence is lost) is 5.5; however between pH 4.5 and 5.5 saliva and plaque fluids are still supersaturated with regard to fluorapatite. This means that fluorapatite will tend to precipitate, preventing the loss of mineral ions. Another important compound formed during topical fluoride treatment is calcium fluoride (CaF_2), which also acts as an important reservoir of fluoride and has an important cariostatic effect (4).

These findings have been discovered predominantly through work carried out in laboratories. How should we apply these findings to determine the effectiveness of the fluoride-containing materials that are developed to prevent demineralization in orthodontic patients today? Should we be undertaking laboratory-based research or clinically based research?

A handsearch of all articles published in four orthodontic journals (American Journal of Orthodontics Dentofacial Orthopedics, the Angle Orthodontist, European Journal of Orthodontics and the Journal of

Orthodontics) over the last two years (January 2008 to November 2009) found four laboratory studies examining the effect of fluoride-containing materials on demineralization around orthodontic brackets and only one clinical study. Have we got this balance right or is it time to re-evaluate how we conduct research in this area?

The relative advantages and disadvantages of investigating the effects of fluoride on the demineralization process in the laboratory compared to investigations carried out in a patient or volunteer have been debated by cariologists for many years (5). I will briefly outline these.

Advantages of Laboratory Studies

Reduced number of variables

One major advantage claimed for laboratory studies is that the number of variables can be strictly controlled and reduced. This lack of confounders simplifies the statistical analysis and interpretation of the results. It will reduce the variability of the data and ensure that a relatively small number of specimens is required in order to detect a statistical significance.

Greater control over the variables leading to demineralization will also improve reproducibility. Experiments can be repeated under comparable conditions and similar results obtained, which again gives the researcher more power to detect a difference between materials.

More sensitive and accurate assessment techniques

Until recently the best assessment techniques, in terms of the ability to detect and measure enamel demineralization and remineralization in an objective and reproducible manner, have led to the destruction of the specimens being examined(6). These techniques have been ideally suited to laboratory studies, where enamel specimens do not need to be reused and can be produced in a relatively quick and reproducible manner. Because these techniques are sensitive to small changes they are able to detect small differences between materials and again this increases the power of studies to detect significant differences.

Ethics and Research Governance

Studies that do not involve human enamel will not generally require the scrutiny and approval of an ethics committee. Obtaining ethical approval can sometimes be a laborious and lengthy process, especially in developed countries such as the UK, where researchers are obliged to abide by quite stringent regulations under the remit of a process called 'Research Governance'.

Time and expense

Many of the laboratory techniques are well-established and relatively straightforward, therefore results can be produced quickly. This will help reduce the burden of the study on the researcher and reduce the costs.

Disadvantages of Laboratory Studies

Not representative of the mouth

It is very difficult to reproduce in the laboratory the conditions that are present in the mouth. Variables will include dietary intake (including fluoride intake), temperature changes, salivary constituents and flow, as well as the susceptibility of the person to caries. Both pellicle and plaque present in the mouth are very difficult to manufacture in the laboratory. The microorganisms that metabolize the fermentable carbohydrate into acids that cause demineralization reside in a very complex structure. Over 1000 different bacterial species have been identified using modern molecular biological techniques. This is about twice the number that can be cultured (7, 8).

Short term

Many laboratory studies are undertaken over a short period of time, usually 28 or 30 days(9, 10). This represents only a small portion of the time that the patient is wearing orthodontic appliances. The effects of the material for the great majority of the treatment period are therefore ignored. In addition the release profile of fluoride from most fluoride-containing materials is of a rapid release in the first few hours and days, followed by a slow, but steady release over a period of time (Figure 1). If materials are only studied when they are first attached to the tooth, including the period of the initial burst of

fluoride released, this may give a false impression as to the effectiveness of the material in preventing demineralization in the long term.

It has been recognized for some time that fluoride-containing materials not only release fluoride, but also absorb fluoride when it is abundant in the mouth, such as after brushing with the fluoridated toothpaste and then release it later (11) (Figure 2). This re-release of fluoride has been shown to occur in the laboratory for up to 30 months(12); however the clinical significance is not clear, as much of the work has been carried out on disks of material that have a much larger exposed surface area than is possible between the bracket and tooth (for an extensive recent review of work in the area of restorative dentistry see Wiegand et al.(13)).

Lack of standardization

There is no standardization in the methodology used in laboratory studies. Demineralization times vary from 48 hours (14) to 96 hours (15). Others have used a pH-cycling technique (16) (see below). Enamel for specimens is harvested from different types of teeth including canines and premolars (17), molars (10), third molars (14) and bovine teeth (16), despite there being evidence of differences in the enamel response to a demineralizing challenge both between the different types of human teeth (18) and between animal species (19).

Interpretation of outcomes

As outlined above the assessment of demineralization has usually involved microscopic techniques that have measured the loss of mineral or depth of demineralization in the outer layer of enamel. Earlier methods, for example polarized light microscopy or microhardness testing assessed demineralization indirectly by comparing measured outcomes with standard measurements collected from specimens with a known mineral content. Newer methods, such as transverse microradiography, use computers to directly calculate the mineral loss of a carious lesion by comparing it to a surrounding area of sound enamel. The most recent techniques, for example quantitative light-induced fluorescence (QLF) and newer microtomography techniques (10, 20) allow measurement without destruction of specimens. This enables the longitudinal determination of enamel mineral content and any subsequent changes occurring with time.

These techniques are powerful, validated methods of determining mineral content that are sensitive to small changes; however the interpretation can sometimes be problematic to the clinician. QLF might detect a difference in mineral content that is statistically significant before the change is visible to the clinician or patient. We also understand that demineralization and remineralization are occurring simultaneously and a small change in diet and/or oral hygiene practices may reverse any detectable change. Is a small change in mineral content therefore clinically significant?

Clinicians (and sometimes patients) are usually more interested in straightforward outcomes such as whether demineralization is present or not. Therefore I would question if very sensitive techniques for measuring mineral loss are an appropriate outcome measure for determining the effectiveness of fluoride-containing materials developed for orthodontic practice? Perhaps taking clinical photographs as a permanent record and assessing them using a dichotomous outcome might be more appropriate? (see Appendix A).

Ways of making laboratory studies more clinically applicable

Ph Cycling techniques

The demineralizing challenge in the mouth is not continuous for 48 or 96 hours at a time, but fluctuates throughout the day. The technique of pH-cycling was therefore described by ten Cate and Duijsters (21) as a way of making laboratory studies more representative of conditions in the mouth. This involves subjecting specimens to a demineralizing solution (pH 4.6 – 4.8) for 30 to 60 minutes six times a day(22). In-between cycles the specimens are stored in an artificial saliva or remineralizing solution(10) to promote repair.

Artificial Mouth Models

Researchers have developed bacterial models designed to simulate oral conditions (23-25). A diagrammatic representation of one model is shown in Figure 3. Other models use a parallel plate flow chamber to simulate a steady flow of saliva over specimens; however this has mainly been used to study plaque biofilms rather than demineralization(26, 27).

Toothbrush wear simulator

A recent study has described a machine that can apply 15,000 brush strokes to a tooth specimen, which represents 20 strokes per day over 2 years. It uses a constant force and provides a continuous recirculating supply of non-fluoridated toothpaste combined with water (15).

Randomized-Controlled Clinical Trials

Advantages of RCTs

Properly conducted clinical trials involving orthodontic patients that are randomly allocated to either receive a fluoride-containing material or a non-fluoridated control material are able to overcome most of the limitations of laboratory studies. The materials are tested in a patient's mouth, which is where they were designed to be used. The experiment is carried out over the full course of orthodontic treatment, not just the first few days or weeks. The effectiveness of the material in preventing demineralization can be assessed by choosing a principal or primary outcome that is relevant to both clinicians and patients, namely did the patient develop any new visible demineralized lesions? This will usually be determined from before and after clinical photographs. A simple statistic that describes the odds of a patient developing a demineralized lesion with the fluoridated material compared with the non-fluoridated control can then be calculated (See calculation of Relative Risk - Appendix A).

The use of this simple dichotomous (yes the patient developed at least one new demineralized lesion/No the patient developed no new demineralized lesions) to determine the incidence of demineralized lesions with one material compared with a control does not exclude the possibility of looking at other secondary outcomes. For example a fluoride-containing material might not reduce the number of new demineralized lesions (incidence) compared with the control material, but may reduce the severity of lesions when they occur. Severity can be measured by examining the area of the lesion, mineral loss or lesion depth. Another important finding for a fluoride-containing bonding material might be to determine if there are more bond or band failures compared with the control. Again this might be assessed using a simple dichotomous outcome (yes the patient had at least one bond or band failure/No the patient had no bond or band failures).

Disadvantages of RCTs

RCTs are time consuming and expensive, both in design and execution. In many developed countries the investigator has to go though long and complicated procedures to obtain approval to carry out the trial. Clinical trials require careful organization and coordination. Patients must be recruited and followed-up through the full course of treatment, with outcomes assessed at key stages.

One important aspect of the design of the study is to recruit sufficient patients in order to detect a significant difference between materials if one truly exists. The size of the sample will depend upon the incidence of the outcome of interest and there are difficulties obtaining relevant and accurate data upon which to base a sample size calculation. The incidence of opacities in patients following orthodontic treatment can be estimated from the literature as between 9 (28) and 26 percent (29). These figures are based on the difference between the prevalence of opacities from clinical examinations of two cross-sectional samples of orthodontic and non-orthodontic individuals. The incidence should ideally be determined by longitudinal examination of individuals before and after orthodontic treatment using a validated technique, such as QLF to distinguish true new demineralized lesions. A recent study using QLF to determine the presence or absence of demineralization in 250 individuals who had recently completed orthodontic treatment found a prevalence of 72 percent. Unfortunately again this was a cross-sectional study therefore it was not clear what proportion of these individuals had actually developed demineralization during orthodontic treatment (30). In fact data for the true incidence of demineralization occurring during orthodontic treatment is lacking, which makes calculation of an accurate sample size for a preventive study difficult. If the incidence is

as low as 9 percent then to detect a meaningful reduction with a fluoride-containing material will require a very large sample size.

One temptation for investigators undertaking a clinical trial in this area is to do a split mouth study (31). This involves using the experimental material for half the teeth in a patients mouth (usually either an upper arch or a lower arch) and the control material for the other teeth. The proposed advantage of this method is that this reduces the number of cofounders such as diet, temperature, salivary constituents and flow, as well as biological susceptibility. If the number of cofounders is reduced then the variability in the data will be reduced and the number of patients that need to be involved in the trial can be smaller. The problem with this approach is that the proposed advantage may work to the disadvantage of an experimental product. The fluoride from the fluoride-containing material may cross the mouth and reduce demineralization on the teeth where the control material was used (32).

Another limitation of some clinical trials is that when they have analyzed their results they have used data based on the demineralization of individual teeth within individual patients. This analysis assumes that one tooth within a person's mouth is independent of the tooth next to it; whereas as we have outlined above this is clearly not the case. Although there are differences in the dynamics of salivary and fluoride flow between different areas of the mouth (32, 33), these are not sufficient to assume statistical independence. Studies that have used tooth surfaces rather than individual patients as the unit of statistical analysis have usually based their sample size on the number of teeth in their study, whereas the effective sample size is the number of patients (or quadrants in the case of split mouth studies), which is actually much lower. These studies are usually under-powered.

Other intra-oral techniques

Due to the cost of randomized controlled trials alternative methodologies to evaluate preventive measures have been suggested (34). This includes the banding or bonding of teeth that are due to be extracted as part of the orthodontic treatment plan (31). Although this seems like a neat solution to the dilemma of clinical studies they still suffer from the shortcomings of limiting the length of time over which the material can be tested, as any delay to extracting the tooth will prolong the orthodontic treatment. Researchers also succumb to the temptation to carry out a split mouth study, which may be to the disadvantage of the fluoride-containing material and also carry out an inappropriate analysis assuming statistical independence of single teeth within a mouth.

Another solution is an *in situ* method whereby a specimen of enamel is attached to the appliance(35) (Figure 4). This can be removed and analyzed at different stages. Treatment is not delayed, but this methodology is quite demanding with respect to laboratory work for the preparation and analysis of the enamel specimens.

Summary

The majority of fluoride-containing materials that have been investigated for their effectiveness in preventing enamel demineralization during orthodontic treatment are bonding agents. Composite resins were developed in the 1960s when Bowen developed the high molecular weight monomer BIS-GMA(36). There have been attempts to add fluoride to composite (37) and early clinical studies showed that these might hold some promise for the prevention of demineralization during orthodontics (38, 39). Later investigations failed to detect significant reductions in the prevalence of demineralization between fluoridated and non-fluoridated composites (40-42). There has been recent interest in fluoridating composites using new methods (43).

Other less commonly used bonding materials are glass ionomer cement, which was developed in the 1970s(44) and polyacid-modified composites or compomers, which became commercially available in 1993. Conventional glass ionomer cement was found to be too weak for orthodontic material, but the strength has been significantly improved by the introduction of resin modified glass ionomer cement (45).

These bonding agents have been commercially available for many years. I would argue that unless there is the discovery of a uniquely new material then orthodontists undertaking research in this field should concentrate their resources on clinical rather than laboratory studies. Cariologists carry out laboratory studies to explaining the mechanisms of action of fluoride. *In vitro* testing is also required

to assess the safety of materials before use in the mouth. As orthodontists we are interested in determining the effectiveness of a product in the prevention of demineralized lesions in our patients. The only way we can determine if a particular fluoride-containing material does reduce demineralization in the orthodontic patient, without any adverse side effects such as bond failures, is to study the material in the environment within which it will be used and for the length of time that it will be used for. This will inevitably mean a randomized controlled trial.

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Figure 1

Mean fluoride release curve for each experimental group: A, control groups (without fluoride mouthrinse); B, test groups (with fluoride mouth rinse). From (16)

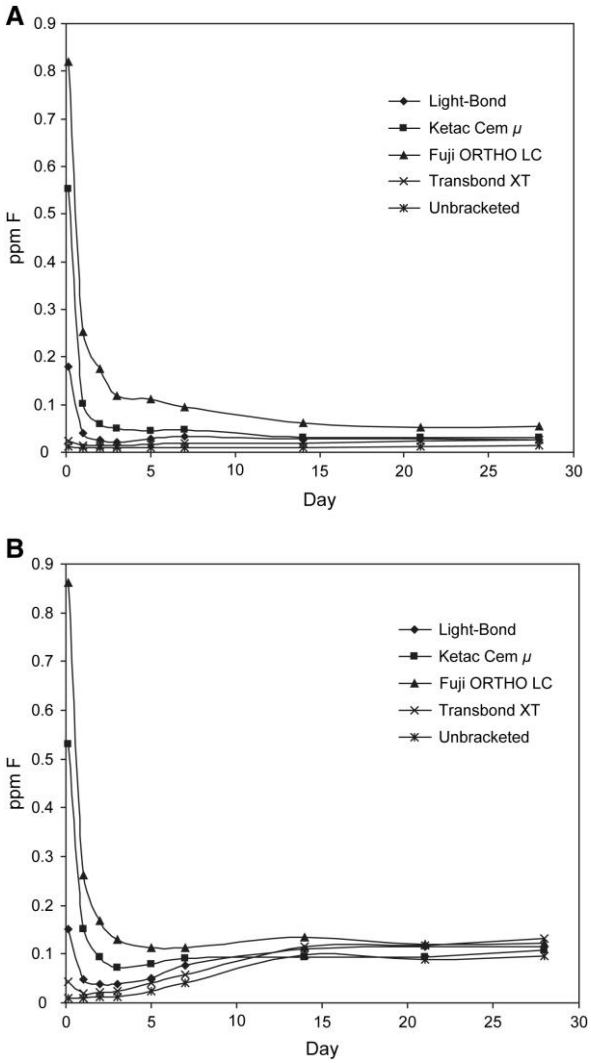


Figure 2

Daily fluoride release of each test material before and after refluoridation. All materials showed an initial burst of fluoride on the first day of water immersion and refluoridation but returned to the baseline 3 or 4 days later (from (11).

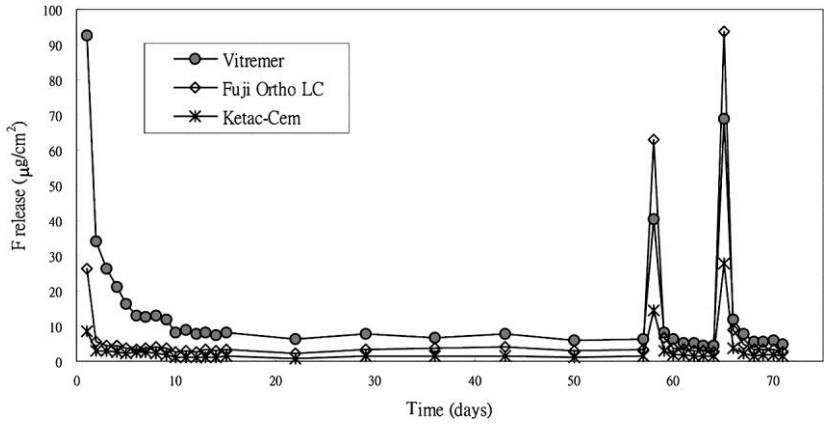


Figure 3

A schematic diagram of the multicell continuous culture bio®lm system reproduced from (24).

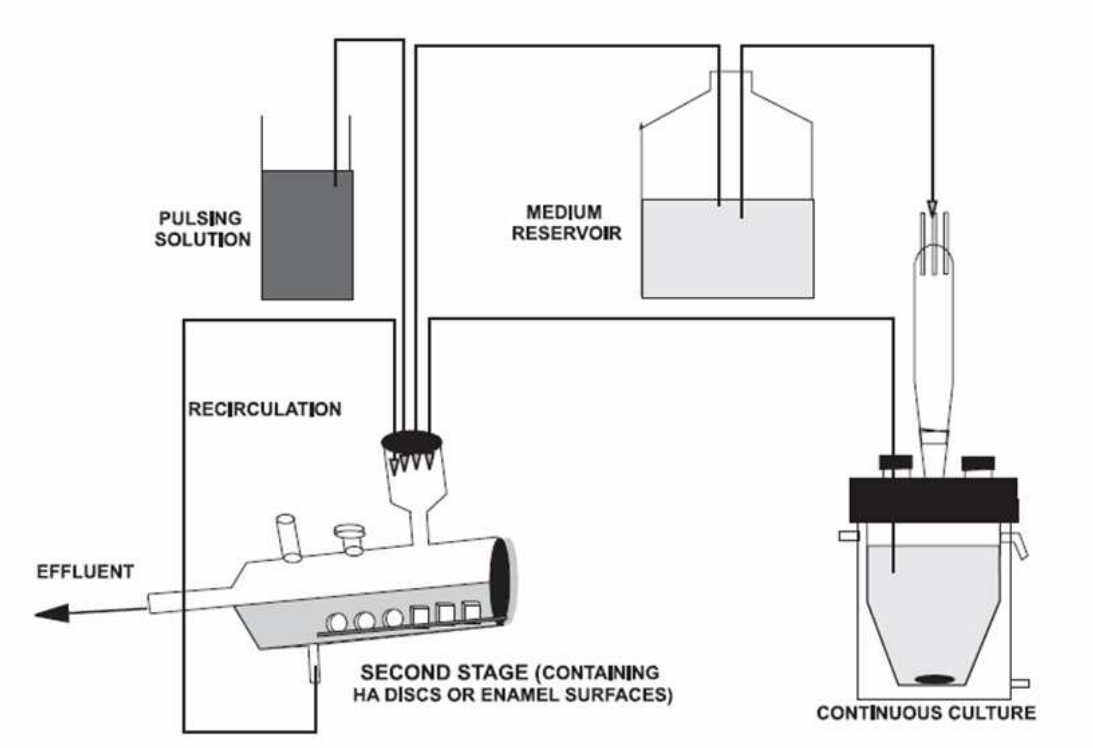
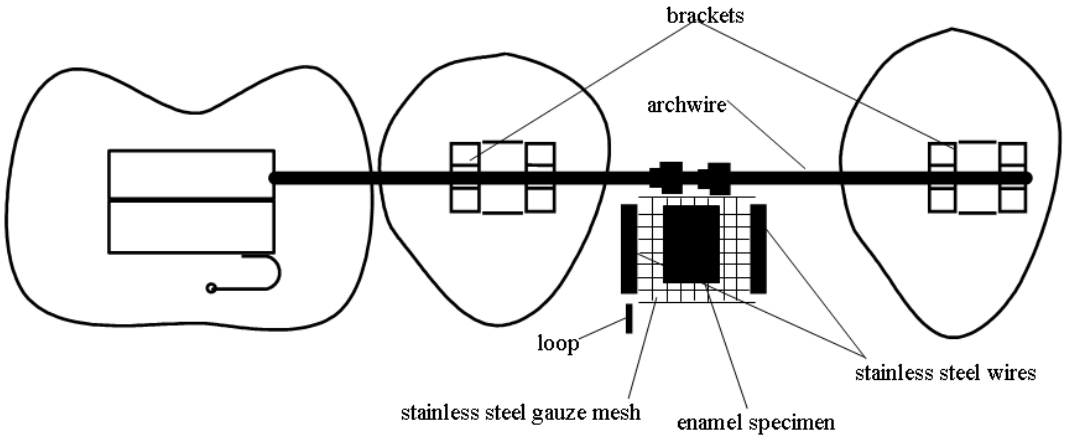
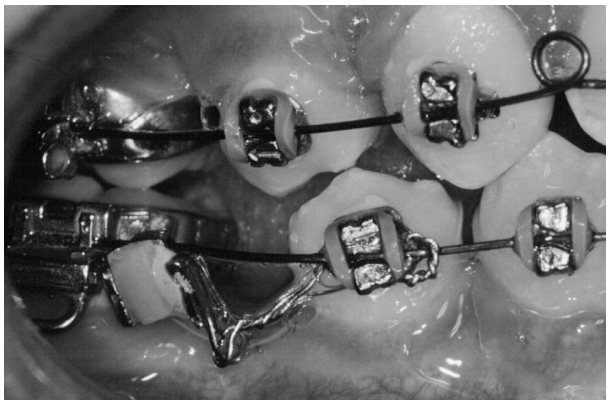


Figure 4

a. Diagram if *in situ* model adapted to the orthodontic environment



b. Clinical picture of fixed appliance with *in situ* enamel specimen within a specially adapted holder placed on the archwire (from (46)).



Appendix A

To assess whether the fluoride-containing experimental material or the non-fluoride containing control material is more likely to have a new demineralized lesion the Relative Risk can be calculated.

A 2 x 2 contingency table is constructed.

		Control material	Experimental material	Total
New demineralized lesion	Yes	*a*	*b*	*a + b*
	No	*c*	*d*	*c + d*
	Total	*a + c*	*b + d*	*n*

Where:

a = the number of patients with control material who had at least one new demineralized lesion

b = the number of patients with experimental material who had at least one new demineralized lesion

c = the number of patients with control material who had no new demineralized lesions

d = the number of patients with experimental material who had no new demineralized lesions

a + b + c + d = the number of patients in the trial.

$$\text{Risk Ratio} = \frac{a/(a+c)}{b/(b+d)}$$

Under the null hypothesis the expected value of RR is 1

Split mouth studies will require a different format. The data should be arranged per quadrants (not individual teeth) as outlined in the table below.

		Fluoride Intervention White Spot Lesions	
Control		Yes	No
White Spot Lesions	Yes	*a*	*b*
	No	*c*	*d*
		a + b + c + d = n	

Where:

a = the number of patients who had at least one new demineralized lesion present in both the experimental and the control arches (or quadrants).

b = the number of patients who had at least one new demineralized lesion present in the control arch, but not in the experimental arch.

c = the number of patients who had at least one new demineralized lesion present in the experimental arch, but not in the control arch.

d = the number of patients who had no demineralized lesions present in either the experimental or the control arches.

If there are two experimental and two control quadrants per individual then ideally the two experiment quadrants and the two control quadrants should be combined. If this is the case then:

a + b + c + d = the number of patients in the trial.

If the data for combined quadrants is not available then it should be possible to use the data for upper and lower quadrants, in which case:

a + b + c + d = twice the number of patients in the trial.

Ideally the data should be for the number of **new** lesions, which were assessed immediately after debond.