An *In situ* Caries Model to Study Demineralisation During Fixed Orthodontics.

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Running Title: An *in situ* model in fixed orthodontics
Key words: demineralisation, orthodontics, microradiography, image analysis.

Acknowledgments

The authors would like to thank Dr. Stephen Rudge, Dr Una Doherty and Dr. Roz Ashwood and their patients for agreeing to participate in this trial, Dr Jayne Harrison for carrying out the randomisation and Dr Simon Fear for statistical advice.

Abstract

The *in situ* caries model is increasingly used to study de/remineralisation *in vivo*. It has a number of advantages over other *in vivo* models. It is non-invasive and does not affect the patient's treatment. The aim of this study was to investigate de/remineralisation of enamel during the early stages of orthodontic treatment using the *in situ* caries model. Fifteen orthodontic patients undergoing fixed appliance treatment with extraction of premolar teeth were included in the study. At the initial placement of the appliance, two enamel samples with pre-formed caries-like lesions were placed bilaterally, in specially constructed holders on the archwire, near the lower extraction site. One sample was bonded with a small bracket base. The side with the bracketed sample was randomly allocated. After one visit (mean 52 days) the samples were removed and two fresh samples were placed. These were removed after two further visits (mean 90 days). Each sample was sectioned and ground to 100μm. The samples were microradiographed and compared, using image analysis, with a control which had not been placed in the mouth. The results showed that there was large variability between individuals. The non-bracketed sample showed statistically significant remineralisation as expressed by the ratio of mineral loss to lesion depth, compared with the control. There was no difference between the dominant and non-dominant sides. The time the sample was left *in situ* could not be correlated with any change in the parameters of the lesion. The *in situ* caries model is a useful method of studying effective regimes that promote remineralisation.

Structured Abstract

Authors – Benson, PE, Pender, N, Higham, SM.

Objectives - To investigate de/remineralisation of enamel during the early stages of orthodontic treatment using the *in situ* caries model.

Design – A prospective, longitudinal study, using the *in situ* caries model.

Setting and Sample Population – The Department of Orthodontics at the University of Liverpool School of Dentistry. Fifteen orthodontic patients undergoing fixed appliance treatment with extraction of premolar teeth.

Experimental Variable – Two enamel samples with pre-formed caries-like lesions were placed bilaterally, in specially constructed holders on an orthodontic fixed appliance. One sample was bonded with a small bracket base.

Outcome Measure – The parameters of the pre-formed carious lesion, expressed as mineral loss (ΔZ), lesion depth (l_d), lesion width (l_w) and ratio ($\Delta Z/l_d$) were compared between the bracketed, the non-bracketed and a control sample which had not been placed in the mouth. The difference between brackets placed on the dominant (toothbrush hand) side and non-dominant side were also investigated. The correlation between mineral loss and length of time the sample was in the mouth was also analysed.

Results – There was considerable individual variation, however a one-factor repeated analysis of variance showed a significant difference in ratio values between the three groups (p=0.006). A pairwise comparison showed a significant reduction in ratio value for the non-bracketed sample compared with the control, but not the bracketed sample. There was no significant difference in mineral loss between the dominant and non-dominant sides. There was no linear correlation between the length of time the sample was in the mouth

Conclusion – An enamel sample with a pre-formed carious lesion, when placed in the mouth of an orthodontic patient showed reduced remineralisation in the presence of a simulated orthodontic bracket. Consistently effective preventive regimes to prevent demineralisation in patients with fixed orthodontic appliances need to be developed. The technique described will be a valuable tool in this process.

Introduction

The development of enamel demineralisation during orthodontic treatment due to inadequate maintenance of appliances is a significant problem. Gorelick *et al* (1) found, in a cross-sectional study, that 50% of individuals undergoing orthodontics had a non-developmental enamel opacity compared with 25% of controls. Øgaard (2) identified that, even five years after treatment, orthodontic patients had a significantly higher incidence of enamel opacities than untreated controls.

Experimental models for the study of orthodontic demineralisation have fallen into two categories:

1. *In vitro* methods - examining the effects of an artificial demineralising solution in a test tube, on a tooth with an orthodontic bracket or band attached (3).
2. *In vivo* methods - attaching a bracket or band to the tooth of a patient, which will be extracted as part of the orthodontic treatment. The tooth is extracted after one month and the surface is examined (4, 5, 6).

The *in vitro* methods have the advantage of attempting to standardise variables present in the mouth, but cannot provide an accurate representation of the true oral environment. The *in vivo* methods are carried out under oral conditions, but have a number of disadvantages. Firstly, it is difficult to obtain adequate controls of unaffected enamel. Secondly, the patient cannot begin their orthodontic treatment until the tooth is extracted. Consequently, the length of the experiment is limited, if the patient's treatment is not to be unduly prolonged. Finally the

experiment is confined to the initial stages of treatment, usually the first month and is unable to monitor changes in the enamel throughout the duration of the treatment.

In situ models have become increasingly utilised in the study of dental caries (7). This method uses a standardised, pre-prepared enamel sample to test demineralisation and demineralisation within the oral environment. It has a number of advantages over other *in vivo* models. The sample of enamel that is placed in the mouth, is taken from a control with an artificial carious lesion. Following a suitable test period, the sample is removed from the mouth. Any change in the parameters of the lesion, either further demineralisation or demineralisation, can be measured and compared with the control, that has not been in the mouth.

The experimental procedures may be carried out at routine appliance adjustment appointments and so do not affect the patient's treatment. The *in situ* model may test conditions in the mouth at various stages of orthodontic treatment. Finally because the experiment does not affect the patient's treatment, each subject may participate in several preventive regimes. They therefore act as their own control, increasing the power of the experiment.

In situ enamel samples have been placed in removable appliances (8), but as far as the authors are aware they have not been used to investigate fixed orthodontic appliances.

The aim of this project was to develop and test the use of the *in situ* caries model in the study of de and remineralisation of dental enamel during orthodontic treatment with fixed appliances. The principal outcome of interest was the difference in the parameters of an artificial enamel lesion among three samples. The first sample was bonded with a small bracket base; the

second was without the bracket, but placed in the mouth of the same individual at the same time. The third was a control that had not been placed in the mouth.

Secondary outcomes of interest were changes in these parameters between the dominant (toothbrushing hand) side and non-dominant side and the change in the parameters with length of time the sample was left *in situ*.

Materials and Methods

Enamel lesions. - The samples were prepared using a technique previously described (9). Premolar teeth extracted for orthodontic purposes were collected and stored in distilled water containing a few grains of thymol. The teeth were carefully examined and those with signs of caries or damage to the enamel were excluded. Selected teeth were lightly abraded with fine abrasive paper to remove the outermost enamel and remnants of the pellicle from the buccal surface. The teeth were varnished with an acid resistant nail varnish except for a window approximately 12 x 2mm on the buccal surface. They were mounted on glass rods using inlay wax and immersed into an acidified gel (0.1M lactic acid, 0.1M sodium hydroxide and 6% hydroxyethylcellulose, pH 4.5) for seven days.

After withdrawal from the gel, the varnish was removed and the block of enamel containing the lesion was cut from the crown of the tooth, together with a margin of sound enamel, which had been under the varnish, above and below the lesion. The lesions were divided to give three sections of approximately 4mm x 2mm in size. One of the sections was retained as the baseline control and the remaining two sections were prepared as if they were to be placed in the mouth. The experimental and control lesions were sterilised by gamma irradiation.

Following sterilisation the control samples were cut perpendicular to the surface and polished to give planoparallel specimens of approximately 100μm thickness. The sections were examined under polarised light microscopy for a subsurface lesion of even quality. Samples with evidence of surface lesions or lesions of poor quality were rejected. The control sections from each acceptable lesion, were placed together with an aluminium step-wedge with 25μm steps, on

high-resolution radiographic film (Kodak, Rochester, NY, USA). They were radiographed in a Phillips X-ray set with a copper target and nickel filter. The exposure time was 18 minutes at 25kV and 10mA. The anode film distance was 30cm.

The microradiograph images were developed and measurement of the lesion parameters carried on a computerised image analysis system (TMRW program version 1.22) using an algorithm developed by de Josselin de Jong *et al* (10). The mineral content of the sections were expressed as mineral loss (Δ Z), lesion depth (l_d), lesion width (l_w) and ratio ($\Delta Z/l_d$) (Fig 1).

The design of the experiment was such that each subject required four samples. Each prepared tooth yielded three sections (two experimental and one control). The subjects therefore required samples from two prepared teeth. The controls of the two samples in each patient were carefully matched according to their mineral loss, as it has been shown that the baseline lesion mineral loss may affect the demineralisation properties of the sample (11).

The experimental enamel lesions were mounted onto customised holders (Fig 2) using a dentine and enamel primer with a light cured composite resin (Prime and Bond/Prismafil, Dentsply De-Trey-Strasse 1, D78467, Konstanz, Germany). The small bracket base, of approximate size 1.5mm x 1.5mm, was constructed from a larger orthodontic molar bracket base (American Orthodontics, 1714 Cambridge Avenue, Sheboygan, WI 53081, USA). It was bonded to the enamel sample with an orthodontic composite resin (Concise, 3M Dental Products, St Paul, MN, USA) according to the manufacturer's instructions. Excess of material was removed from around the bracket edges with a sharp probe. A small bracket base was used to simulate the environment of an orthodontic bracket, but without the bulk.

Subjects and experimental procedures. - Ethical approval for the study was obtained from the local Ethical Committee. The subjects for the trial were selected from those individuals about to undergo fixed orthodontic treatment in the Orthodontic Department of Liverpool Dental Hospital and who required orthodontic extractions as part of their treatment. The latter requirement was to ensure there was enough space to place the customised holders with the enamel sample (Fig 2). Written consent of all patients and parents agreeing to participate was obtained.

Statistical advice concerning sample size was obtained. Data from a previous experiment suggested a clinically relevant mineral loss of 300vol%.μm with a standard deviation of 200vol%.μm would give us sufficient power using 10 patients assuming a paired t-test with $\alpha = 0.05$. It was decided to recruit fifteen individuals to allow for loss of samples or withdrawals from the study. The fifteen individuals consisted of nine females and six males. The median age was 13.5 years (range 12.3 years to 38.8 years).

The study was designed so that each patient acted as his or her own control. Each patient received four enamel sections. Two sections were placed at the start of treatment when the first archwire was placed. These were removed at the first adjustment visit (mean 52 days) and two new enamel sections were placed. These remained in the mouth for two adjustment appointments (mean 90 days).

The samples were placed in pairs. On the surface of one sample in each pair was bonded a small bracket base to mimic the environment of the conventional orthodontic bracket. The other sample had no bracket. The customised holder was placed over the archwire of the lower

orthodontic appliance in the extraction site (Fig 2). It was secured with a stainless steel ligature, to prevent rotation. One bracket was placed in the left extraction site and one in the right.
The side containing the sample with the small bracket base was randomly allocated by a block randomisation technique to either be placed on the dominant (or toothbrushing hand) side or on the non-dominant side. All patients were instructed in the use of a fluoride toothpaste and fluoride mouthrinses.

Measurement of de- and remineralisation. - Following removal from the mouth the samples were taken off the customised holders with an orthodontic debonding instrument. The small bracket base was carefully removed from the bracketed samples. The enamel was stored in distilled water, before preparing for microradiography. During the preparation the samples were cut perpendicular to the surface and polished to give between two to four plano-parallel sections of approximately 100μm thickness. After preparation the samples were recoded by a second investigator to allow blind assessment by the principal investigator. Each patient had six samples (four experimental and two controls). All six samples were microradiographed on the same film, together with the calibrating stepwedge, to minimise random error due to problems with exposure and developing. The microradiographs were quantified by computerised image analysis. The parameters of the lesions, expressed as mineral loss (ΔZ), lesion depth (l_d), lesion width (l_w) and ratio ($\Delta Z/l_d$) were compared by statistical analysis (see below).

To investigate the reproducibility of the technique, five radiographs containing 30 samples were re-coded for a second blind assessment by the principal investigator two weeks after the first assessment.

Statistical Analysis. - Each sample was cut into between two to four sections depending on the size of the original sample. All the sections were examined and a total of between three and five readings were taken to obtain a representative reading for that sample. The mean of these readings was then chosen for statistical analysis. All statistics were carried out using SPSS for Windows (SPSS Inc., 444 Michigan Avenue, Chicago, Il. USA)

Reproducibility . - The index of reliability was calculated and a one sample t-test carried out on the repeat readings to assess random and systematic error (12).

Hypothesis testing. - The null hypothesis was that there is no difference in the changes to the lesion parameters amongst the three samples. The data was examined graphically and tested with a Shapiro-Wilk statistic to assess whether it had a Normal distribution. On two occasions the data was found to be skewed and was transformed to a Normal distribution. Hypothesis testing was carried out with a one-factor repeated analysis of variance. Multiple comparisons were carried out with a paired t-test correcting for type I error by using the Bonferroni t (13).

To test the change in the parameters with length of time the sample was left *in situ*, the percentage change in the respective variables was calculated (11). This was carried out by dividing the sample value by the control value and multiplying by 100 (thus a value of 100 would signify no change, less than 100 would signify remineralisation and more than 100 would signify further demineralisation). Scatter plots were prepared of the change in the variable against the number of days the sample was in the mouth to examine for any association. The Pearson's product moment correlation coefficient was calculated to assess for any linear association.

Results

Fifteen patients were recruited for this investigation. One patient withdrew in the early stages, as he was unable to tolerate the intra-oral carrying device. Of the 56 samples placed in the mouth four samples, from two patients, were lost due to fracture of the archwires. Two samples, from one patient, were lost in processing. No samples were lost due to debonding of the sample from the bracket. Thus a total of 50 samples (25 with the simulated bracket and 25 without the simulated bracket) from 14 patients were analysed. Fifteen of the bracketed samples and ten of the non-bracketed samples were placed on the same side as the dominant toothbrushing hand. Conversely ten of the bracketed samples were placed on the non-dominant side and fifteen of the non-bracketed samples.

The results of the reproducibility assessment are given in Table 1. The index of reliability provides an indication of the proportion of the total error that is due to random error (12). If the random error is a large proportion of the total variability, a result that would have been significant without error may become non-significant (a Type II error). In this study the proportion of random error did not exceed 10 percent of the total variability.

The t-tests for systematic error (Table 1) showed that for the lesion width and ratio there was no evidence of a systematic error between the first and second reading. However for the mineral loss and lesion width there was evidence of systematic error at the 5% level. In both readings the second recording was lower than the first. The variability of these readings was such as to suggest that this was unlikely to be responsible for a Type II error.

Tables 2 and 3 show the means, standard deviations, confidence intervals of the means and the ranges of the four parameters. Table 2 refers to the control, bracketed and non-bracketed samples. Table 3 refers to the control, dominant and non-dominant samples.

These descriptive statistics indicate that there was a trend toward reduction in mineral loss and ratio values in both the bracketed and non-bracketed samples, but this reduction was greater in the non-bracketed sample. The depth and width of the lesions did not show a similar reduction. The results were similar to the dominant and non-dominant statistics, the dominant samples showing the greater reduction. There was however a large variation both between and within individuals.

The analysis of variance between control, bracketed and non-bracketed samples (Table 4) showed a statistically significant difference ($p=0.006$) between the ratio values. The pairwise comparisons corrected for a type I error showed (Table 5) a significant difference between the control and the non-bracketed sample.

The analysis of variance between control, dominant and non-dominant samples (Table 6) also showed a statistically significant difference ($p=0.013$) between the ratios of the three groups. The pairwise comparisons (Table 7) showed a significant difference between the control and the dominant sample and the control and the non-dominant sample, but no difference between the dominant and non-dominant samples.

Figure 3 shows a typical scattergram of the results of percentage change in mineral loss against the time the sample was in the mouth. No association is apparent. Table 8 shows the

Pearson’s product moment correlation coefficients. There were no linear associations between change in any of the parameters with time.

Discussion

The aim of the present study was to investigate whether, within the oral environment of an orthodontic patient, a sample of demineralised enamel containing a simulated orthodontic bracket was at risk of further demineralisation, compared with a sample without a simulated bracket. Examination of the results on an individual basis demonstrated great variability both between and within patients. The overall trend was for remineralisation of both bracketed and non-bracketed samples; however only the non-bracketed sample showed a statistically significant reduction in the ratio compared with the control.

The ratio is calculated by dividing the estimated mineral loss by the lesion depth (Fig 1). Arends *et al* (14) consider the ratio to be an important parameter. They state that the ratio corresponds to the average amount of mineral that is absent or has been lost in a section, therefore it also represents the average amount of mineral loss from an average enamel prism. The caries process starts with diffusion of mineral from the prism periphery (15). Small ratio values suggest loss of interprismatic mineral, whereas large ratio values suggest loss from the prism surfaces, breakdown of prism structure and cavitation.

The results of this study show that the average mineral loss was significantly lower for the non-bracketed sample than for the control. This suggests that there was significant remineralisation for the non-bracketed sample. There was no difference between the average mineral lost between the control and the bracketed sample, although there was notable individual variation and in a few cases there was significant further demineralisation. The largest average mineral loss ratio

for a bracketed sample was 24 vol%. This is well below the figure of 36 vol% which Arends *et al* (14) suggest is the point at which there may be collapse of the prism structure and cavitation.
In the present study we were interested in examining the conditions that were present in a representative group of patients with fixed appliances. All patients were instructed in the use of a fluoride toothpaste and mouthwash, however neither was provided and no attempt was made to measure compliance. The reduction of demineralisation during orthodontic treatment by the use of fluoride has been demonstrated (16, 17). The advantage of the *in situ* caries model is that both samples were tested, at the same time, under the same conditions. If the patient did not comply with the mouthwash instructions then both samples would be equally affected. It was the difference between the two samples that was of interest, as well as the difference between these samples and a control sample that had not been placed in the mouth. This study found that although there was considerable individual differences there were no statistically significant differences between the bracketed sample and the control.

The *in situ* method has the advantage of a control with an artificial carious lesion with measurable parameters. The sample parameters can be directly compared with the control and de/remineralisation can be measured directly. O'Reilly and Featherstone (4) using the *in vivo* method assumed that the mineral content of the enamel was 85 volume percent mineral. They postulated that any decrease in the mineral content was due to demineralisation and any increase due to remineralisation.

O'Reilly and Featherstone (4) found a mineral content below 85 volume percent mineral in the surface enamel beneath the bracket, which they postulated, was due to mineral loss from the acid etching. If this is the case our bracketed sample may have had more mineral loss than the control

when it was placed in the mouth and therefore the control could not be considered valid for the bracketed sample. We thought it necessary to acid etch the bracketed sample for two reasons. Firstly we carried out a pilot study to investigate the possibility that acid etching leads to significant mineral loss (18). The results suggested that although there may be a small amount of mineral loss with the acid etch technique, this was not significant enough to be detected with our technique and therefore the control could still be considered valid for the bracketed sample. We did not find this result surprising, because all the samples had been subjected to a demineralising environment for seven days and we did not consider that a 30 second etch would increase mineral loss significantly. Secondly, we were interested in comparing the effects of a sample of enamel that had undergone all the procedures involved in placing an orthodontic bracket with a sample of enamel that had not. Acid etching is a routinely performed in orthodontics and the comparison would not be clinically valid without it.

Investigation into the affect of dominant versus non-dominant placement of the samples showed no difference between the two sides. There was a significant reduction in average mineral loss whether the sample was placed on the dominant toothbrushing side or the non-dominant side.

Examination of the effect of length of time the sample was left *in situ* with any of the parameters measuring de/remineralisation showed no relationship. This is contrary to the findings of Øgaard *et al* (8). They used the orthodontic banding model to looked at demineralisation when the band was left for 4, 6 or 8 weeks. They found an approximately linear relationship between enamel demineralisation and the time the band was left *in situ*. The orthodontic banding model has been discussed elsewhere. This is an excellent model for the study of demineralisation under a loose or poorly fitting orthodontic band. It will not accurately represent the environment of a bracket,

which may be subjected to intermittent cleaning. The results of this study would suggest that enamel is at risk of demineralisation any time during orthodontic treatment. The clinician must therefore be vigilant throughout treatment in monitoring the patient for signs of demineralisation.

In summary, this study has shown that the *in situ* caries technique can be used as a model to investigate demineralisation with fixed orthodontic appliances. It has a number of advantages over other *in vivo* techniques. It was found that when orthodontic patients were given instructions in the use of a fluoridated toothpaste and mouthwash, there was no increase in the demineralisation of an artificial enamel lesion with a simulated orthodontic bracket, compared with a control. However the bracketed sample showed reduced remineralisation in the oral environment compared with a similar enamel sample without a simulated bracket and in some cases further demineralisation was seen.

Orthodontists should not be complacent about the potential harm appliances may cause if they are not well maintained. Consistently effective preventive regimes to prevent demineralisation in patients with fixed orthodontic appliances need to be developed. The technique described will be a valuable tool in this process.

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Legends

Tables

Table 1 – Descriptive and reproducibility statistics for the repeat measurement of mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%) in 30 samples.

Table 2 - Means, standard deviations, confidence intervals and ranges for control, bracketed and non-bracketed samples parameters of mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%).

Table 3 - Means, standard deviations, confidence intervals and ranges for control, dominant and non-dominant samples parameters of mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%).

Table 4 - Results of one factor repeated measures analysis of variance for mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%) between control, bracketed and non-bracketed samples and control, dominant and non-dominant samples (n=25).

Table 5 - Results of paired t-tests and Bonferroni t between control, bracketed and non-bracketed samples (n=25) and control, dominant and non-dominant samples (n=25).

Table 6 - Pearson's product moment correlation coefficients (r) to assess linear correlation of percentage change in parameter with time for mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%).

Figures

Figure 1

Diagram of a transverse microradiography plot showing the measurements used to calculate the parameters of mineral loss (ΔZ), lesion depth (l_d) and lesion width (l_w).

Figure 2

Diagram of the first enamel specimen holder designed for the pilot study 1.

Figure 3

Diagram of the enamel specimen holder design for pilot study 2.

Figure 4

Image of customised *in situ* enamel specimen holder with enamel specimen and orthodontic bracket base.

Figure 5

Scattergram of change in ratio with time for bracketed specimens (r=0.181)

TABLE 1

Descriptive and reproducibility statistics for the repeat measurement of mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%) in 30 samples.

	Mean Difference	SD	95% Confidence Interval	Index of Reliability	T-Test Significance
ΔZ (vol%.μm)	51.8	113.3	10.5 – 62.0	93.4	2.46*
L_d (μm)	2.0	4.8	0.2 – 3.8	91.1	2.21*
L_w (μm)	1.3	4.3	-0.2 – 2.9	92.4	1.67
Ratio (vol%)	0.37	1.4	-0.2 – 0.9	93.2	1.37

*$p<0.05$

TABLE 2

Means, standard deviations, confidence intervals and ranges for control, bracketed and non-bracketed samples parameters of mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%).

Parameter	Statistic	Control (n=25)	Bracket (n=25)	Non-bracket (n=25)
ΔZ (vol%.μm)	Mean	792.4	764.8	734.7
	sd	302.9	392.6	414.4
	95% Confidence Intervals	667.7 - 917.4	602.7 - 926.9	563.7 - 905.8
	Max	1400.3	1676.5	1809.3
	Min	397.5	362.4	176.75
L_d (μm)	Mean	49.6	52.8	53.2
	sd	7.9	11.8	13.7
	95% Confidence Intervals	46.9 - 52.9	47.9 - 57.7	47.5 - 58.8
	Max	67.1	77.9	78.4
	Min	34	34.8	23.4
L_w (μm)	Mean	39.0	39.1	39.1
	sd	8.5	12.6	12.6
	95% Confidence Intervals	33.6 - 42.5	33.9 - 44.3	33.9 - 44.4
	Max	57.6	67.2	66.8
	Min	22.9	21.6	14.4
Ratio (vol%)	Mean	15.7	14.0	13.4
	sd	4.4	4.3	4.7
	95% Confidence Intervals	13.9 - 17.5	12.2 - 15.8	11.4 - 15.3
	Max	23.8	23.8	24.2
	Min	9.9	8.3	6.4

TABLE 3

Means, standard deviations, confidence intervals and ranges for control, dominant and non-dominant samples parameters of mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%).

Parameter	Statistic	Control (n=25)	Dominant (n=25)	Non-dominant (n=25)
ΔZ (vol%.μm)	Mean	792.4	732.3	767.2
	sd	302.9	361.6	441.5
	95% Confidence Intervals	667.4 - 917.4	583.0 - 881.5	585.0 - 949.5
	Max	1400.3	1676.5	1632.5
	Min	397.5	181.5	176.8
L_d (μm)	Mean	49.6	52.9	53.1
	sd	7.9	11.9	13.6
	95% Confidence Intervals	46.3 - 52.9	48.0 - 57.8	47.5 - 58.7
	Max	67.1	77.9	78.4
	Min	34.0	23.4	27.4
L_w (μm)	Mean	39.0	38.5	39.6
	sd	8.5	11.6	13.5
	95% Confidence Intervals	33.6 - 42.5	33.8 - 43.3	34.1 - 45.2
	Max	57.6	65.8	67.2
	Min	22.9	14.4	18.3
Ratio (vol%)	Mean	15.7	13.6	13.8
	sd	4.4	3.8	5.1
	95% Confidence Intervals	13.9 - 17.5	12.0 - 15.2	11.7 - 15.9
	Max	23.8	23.5	24.2
	Min	9.9	7.7	6.4

TABLE 4

Results of one factor repeated measures analysis of variance for mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%) between control, bracketed and non-bracketed samples and control, dominant and non-dominant samples (n=25).

Samples	Parameter	F-statistic	Significance
bracketed v non-bracketed	ΔZ	1.39	0.258
	L_d	0.91	0.409
	L_w	0.02	0.976
	Ratio	5.74	0.006**
dominant v non-dominant	ΔZ	0.10	0.903
	L_d	0.84	0.438
	L_w	0.13	0.882
	Ratio	4.75	0.013*

* $p<0.05$

** $p<0.010$

TABLE 5

Results of paired t-tests and Bonferroni t between control, bracketed and non-bracketed samples (n=25) and control, dominant and non-dominant samples (n=25).

Samples	t-statistic	Critical t	Significance
control v bracket	-2.32	2.57	
control v nonbracket	3.17	2.57	*
bracket v nonbracket	1.10	2.57	
control v dominant	-2.82	2.57	*
control v nondominant	-2.85	2.57	*
dominant v nondominant	0.88	2.57	

* significant Bonferroni t

TABLE 6

Pearson's product moment correlation coefficients (r) to assess linear correlation of percentage change in parameter with time for mineral loss (vol%.μm), lesion width (μm), lesion depth (μm) and percentage mineral loss (vol%)

	Parameter	Correlation Coefficient (r)	Significance
bracketed v time	ΔZ	0.293	0.156
	L_d	0.186	0.373
	L_w	0.125	0.553
	Ratio	0.181	0.386
non-bracket v time	ΔZ	0.022	0.915
	L_d	-0.086	0.683
	L_w	-0.176	0.401
	Ratio	0.126	0.550
dominant v time	ΔZ	0.120	0.567
	L_d	0.038	0.856
	L_w	-0.096	0.647
	Ratio	0.178	0.393
non-dominant v time	ΔZ	0.182	0.383
	L_d	0.024	0.911
	L_w	0.007	0.972
	Ratio	0.136	0.517

FIGURE 1

Diagram of a transverse microradiography plot showing the measurements used to calculate the parameters of mineral loss (ΔZ), lesion depth (l_d) and lesion width (l_w).

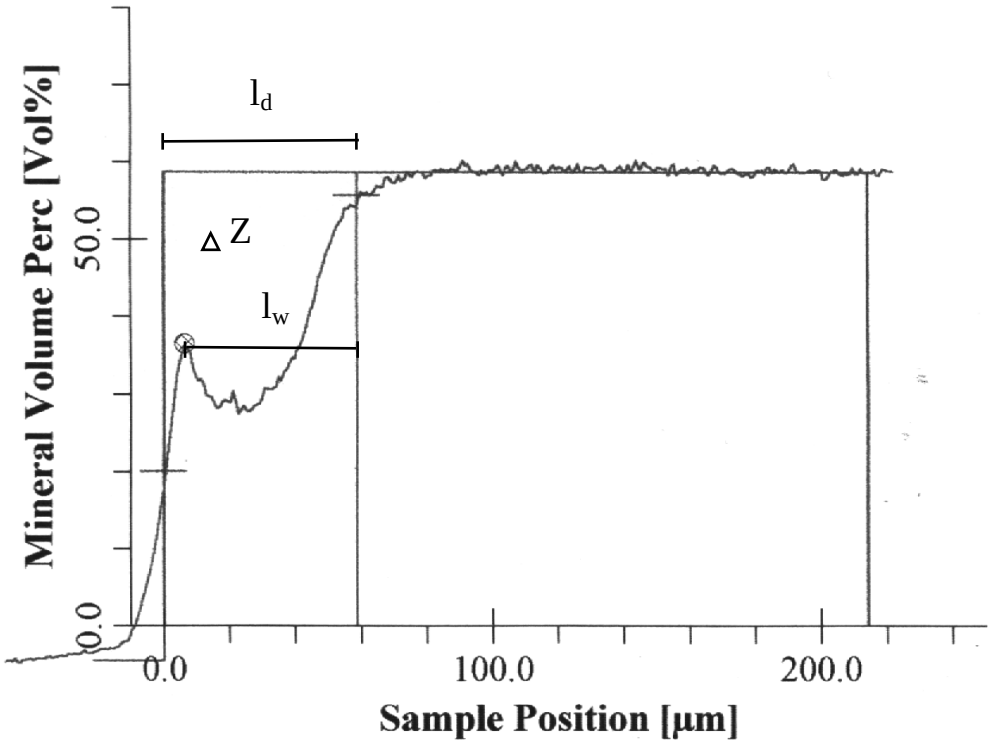

FIGURE 2

Diagram of the first enamel specimen holder designed for the pilot study 1.

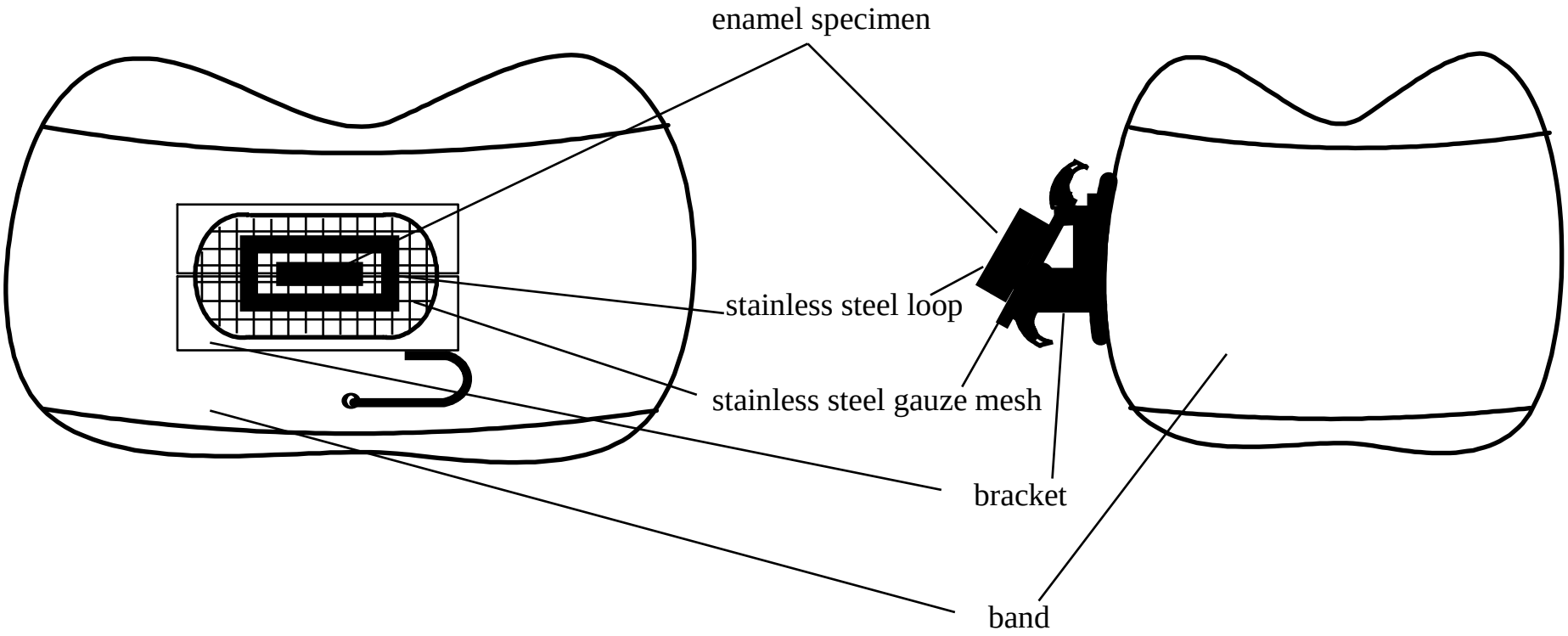

FIGURE 3

Diagram of the enamel specimen holder design for pilot study 2.

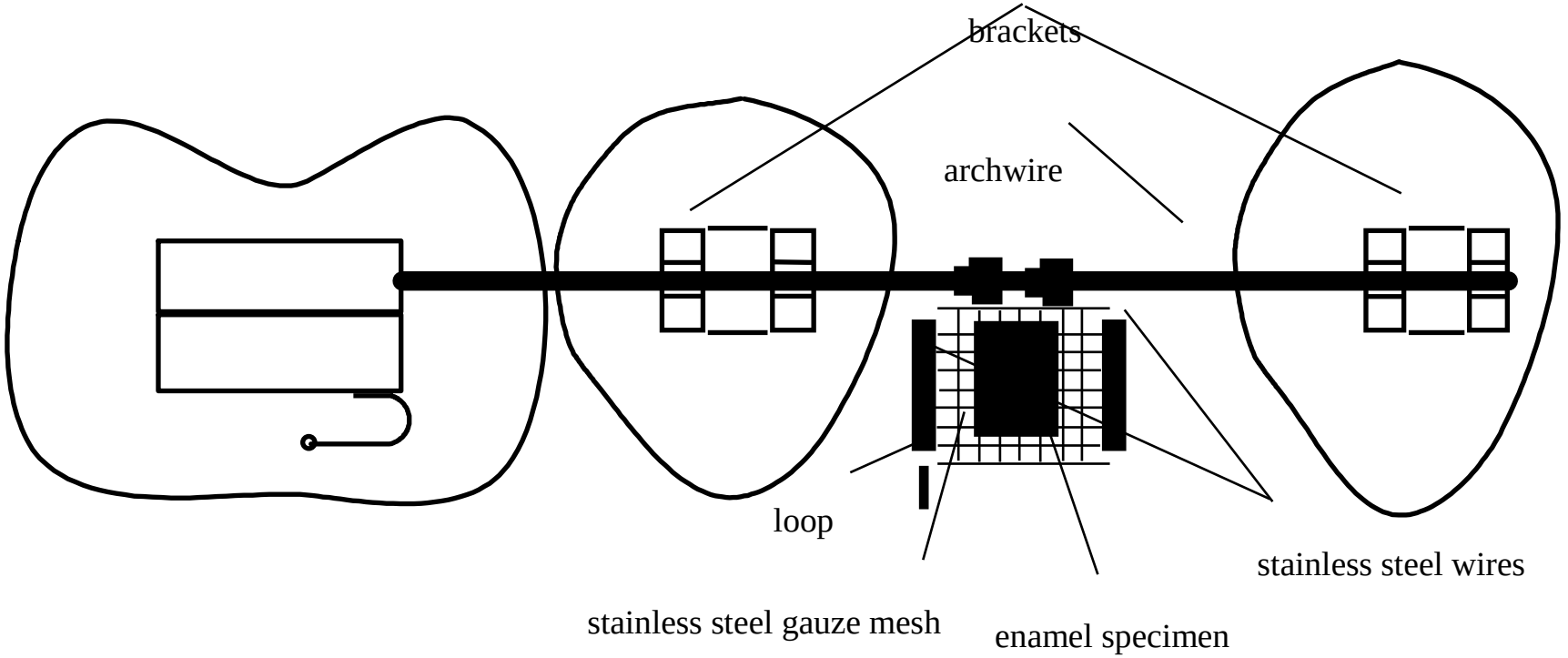

FIGURE 4

Image of customised *in situ* enamel specimen holder with enamel specimen and orthodontic bracket base.

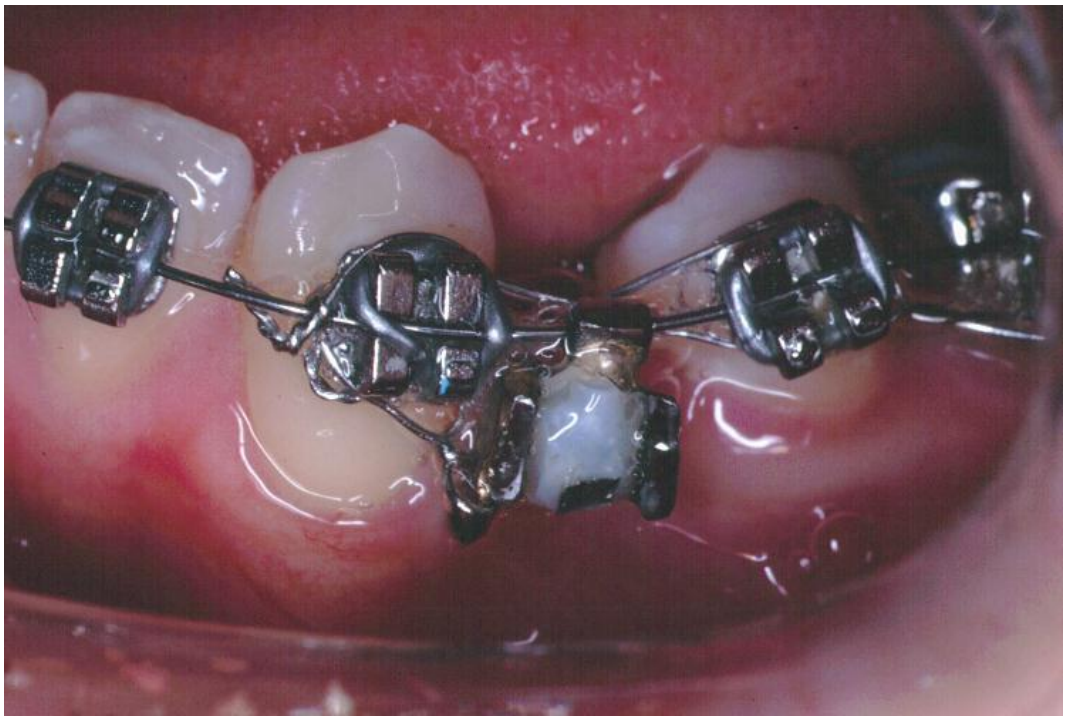

FIGURE 5

Scattergram of change in ratio with time for bracketed specimens (r=0.181).