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The *in vitro* detection of early enamel de- and re-mineralization adjacent to bonded orthodontic cleats using quantitative light-induced fluorescence

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SUMMARY The purpose of this study was to determine whether quantitative light-induced fluorescence (QLF) could detect very early demineralization and remineralization longitudinally adjacent to orthodontic components in an *in vitro* model. Extracted human premolars (n = 13) were sectioned sagittally to produce two equal halves and an orthodontic cleat was bonded to the buccal surface of each tooth. Transparent nail varnish was placed over the remaining surface, leaving exposed enamel windows adjacent to the cleat on the coronal and gingival aspects. Each half-tooth was placed into the lid of an Eppendorf tube and randomly assigned to either control (distilled water) or experimental (lactic acid demineralizing buffer, pH 4.5) regimes. Digital photographs and QLF baseline images were taken. The tubes were mounted into a rotating holder and left for 24 hours. QLF and digital photographs were taken, the solutions refreshed and the teeth returned. This was continued every 48 hours for 288 hours. At this time the lactic acid buffer was replaced with a remineralizing solution (artificial saliva, fluoride, calcium) and the experiment continued with weekly examinations. QLF images were analysed and ΔQ at the 5 per cent threshold recorded.

Analysis of the QLF images showed that both demineralization and remineralization were identified and monitored. Statistical differences between each of the timed examinations were found ($P < 0.05$). Analysis of the photographs demonstrated that QLF detected subclinical lesions. This initial pilot study has demonstrated the potential for QLF to longitudinally monitor de- and re-mineralization of enamel adjacent to orthodontic cleats *in vitro*.

Introduction

The elective nature of most orthodontic procedures and the need to reduce harmful side-effects from such therapies has led to an interest in the detection and prevention of enamel demineralization adjacent to orthodontic components (Øgaard *et al*., 1988a,b; Øgaard, 1989; Øgaard and Rølla, 1992; Ng'ang'a and Øgaard, 1993; Dubroc *et al*., 1994; Jordan, 1998; Vorhies *et al*., 1998; Marini *et al*., 1999; Wilson and Donly, 2001). *In vitro* methodologies for the study of enamel demineralization typically employ artificial lesions that are subsequently analysed using polarized light microscopy, transverse microradiography (TMR), clinical visualization, stereomicroscopy, and electron microscopy (Ghani *et al*., 1994; Øgaard and Ten Bosch, 1994; Melrose *et al*., 1996; Øgaard *et al*., 1996; Benson *et al*., 1999). However, while proof-of-concept trials *in vitro* are acceptable, current research standards suggest that blinded clinical trials offer the best evidence. In order to achieve this, a method for detecting, quantifying, and longitudinally monitoring enamel demineralization *in vivo* is required. Quantitative light-induced fluorescence (QLF) may provide such a method (van der Veen and de Josselin de Jong, 2000).

Quantitative light-induced fluorescence (QLF)

QLF is an optical, visual light-based detection and quantification system for assessing early demineralization of human enamel (van der Veen and de Josselin de Jong, 2000), comprising a hand-held intra-oral camera, an external light source, and a computer (see Figure 1). The basis of the technique is that, under defined conditions, human enamel will auto-fluoresce. Demineralized enamel will result in a reduction of this fluorescence with respect to surrounding sound enamel (de Josselin de Jong *et al*., 1995; van der Veen *et al*., 1997, 1998). This difference in fluorescent intensities enables the degree of demineralization to be quantified and, with several images of the tooth taken over time, longitudinally monitored to assess lesion activity (van der Veen and de Josselin de Jong, 2000). An example of an early enamel lesion under QLF conditions and the resultant analysis is shown in Figure 2. A predecessor of QLF, quantitative laser-induced fluorescence has been applied to *in vivo* orthodontic patients. A study by Al-Khateeb *et al*. (1998) examined active carious lesions in young orthodontic patients revealed after removal of brackets and bands. With a 1-year follow-up, the study found that remineralization of the lesions had occurred. QLF has

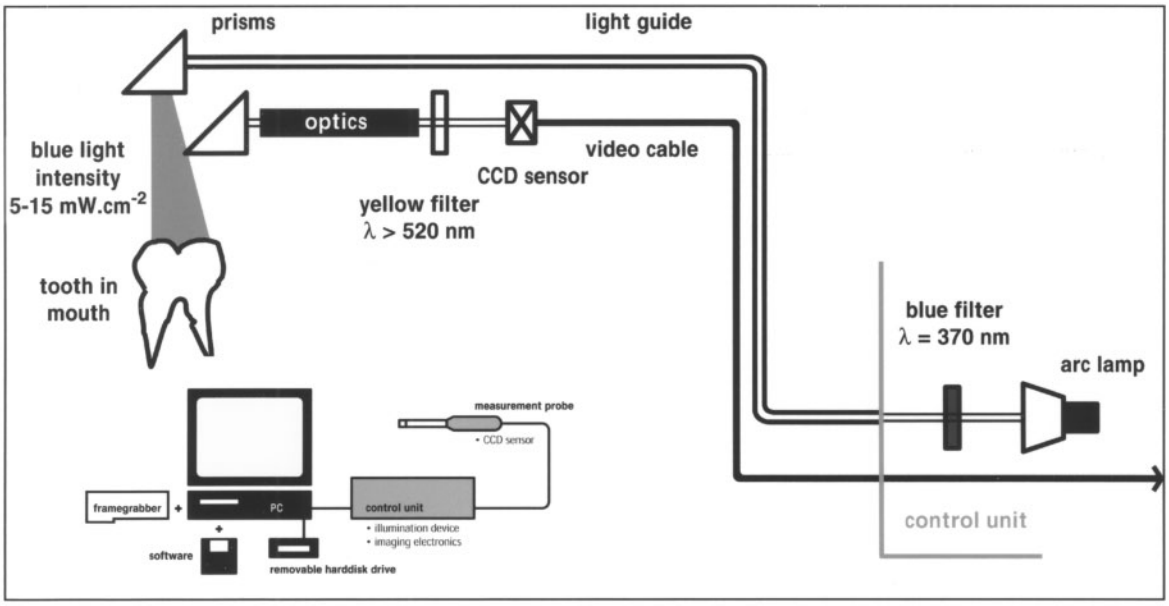

Figure 1 Diagrammatic representation of the QLF device.

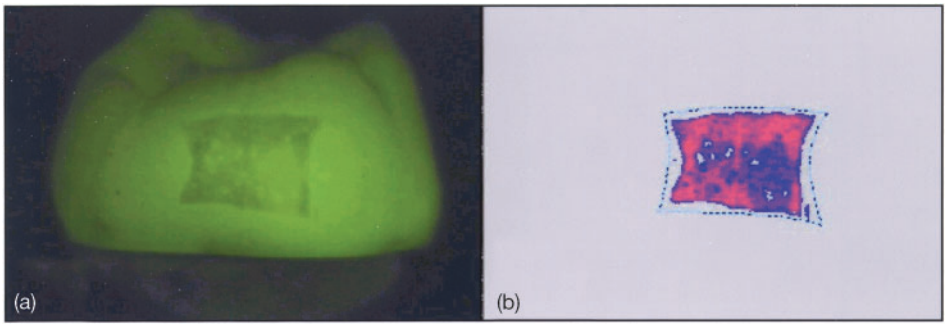

Figure 2 (a) An example of an artificial carious lesion on the buccal surface of a human molar. Note the loss of fluorescence associated with the lesion compared with the surrounding sound enamel. (b) An example of lesion analysis.

been validated against a number of established methods for the quantification of demineralization, including TMR, polarized light microscopy and other optical techniques such as DIAGNODent (de Josselin de Jong, 1995; Ando *et al*., 1997a,b; van der Veen *et al*., 1997; van der Veen and de Josselin de Jong, 2000; Shi *et al*., 2001).

The aim of the current study was to determine whether QLF can longitudinally detect de- and re-mineralization adjacent to orthodontic components in an *in vitro* system. In this way the system's potential for *in vivo* use can be assessed.

Materials and methods

Tooth preparation

Thirteen previously extracted human premolars were selected based upon their clinical appearance as free from stain, caries, enamel defect, or restoration. Each tooth was then examined using the DIAGNODent (KaVo, Bucks, UK) to ensure that no early carious lesions were missed visually. Subsequently the teeth were examined with QLF to ensure that no loss of fluorescence was detected at this stage. Each tooth was then gently pumiced (SS White, London, UK) and abraded with wet-and-dry paper. The anatomical crown was then sectioned from the root using a diamond blade (Walter Ebner, Basel, Switzerland) and the root portion discarded. Each crown was then sagittally sectioned in two using a diamond wire (Well, Walter Ebner). Each crown half was placed in the lid of a 2.0 ml Eppendorf tube (Eppendorf, New York, USA) and randomly allocated to either the control or experimental group, ensuring that 50 per cent of the experimental teeth were distal halves.

To give clinical relevance to the model system, each tooth half was bonded so that half the cleat covered that part of the buccal surface which is normally occupied by

bonded attachments. To ensure that no flash surrounded the bonded cleat, and that no etchant escaped from the bonding area, a mask was used. Briefly, a bi-colour adhesive shape measured to the size of the cleat base was placed on the buccal surface at the site of bonding (Figure 3a) so that the remainder of the surface could be varnished. The adhesive strip was removed and etching with a 37 per cent phosphoric acid gel was confined to the bonding area. After rinsing and drying the cleat was positioned with TransBond (3M, San Francisco, USA) and all flash removed before using a 450 nm curing light to polymerize the bonding material and bond the cleat in place. All varnish was then removed from the buccal surface.

Acid-resistant, transparent varnish was then re-applied across the remainder of the tooth surface leaving two exposed enamel windows, one inferior and one superior to the borders of the cleat. This created four zones on the tooth surface, v, the cleat, w, the area covered by varnish, and x and y, the exposed enamel windows (see Figure 3b). Baseline QLF and white light digital images were taken and stored on a personal computer.

Demineralization

The lids containing the tooth samples were then attached to centrifuge tubes each of which contained either 1.5 ml of demineralizing solution (2.2 mM KH_2PO_4, 50 mM acetic acid, 2.2 mM of $CaCl_2$, 0.5 ppm fluoride at pH 4.5) for the experimental tooth samples, or distilled water for the control samples. The tubes were gently agitated using a blood tube rotator. Initially after 24 hours (and then every 48 hours) for 288 hours the lids were removed from the centrifuge tubes, a QLF and white light image taken, and then the lids were replaced onto a tube containing fresh solution.

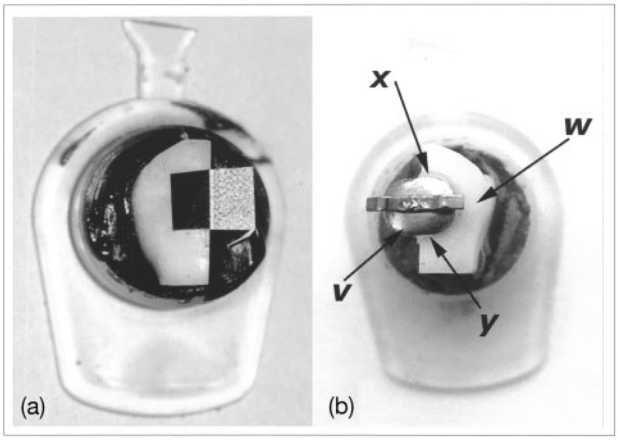

Figure 3 (a) A bi-colour adhesive tape was applied to the enamel surface. This ensures that the varnish and etchant are restricted to specific zones. (b) A premolar prepared for the study with a bonded cleat. Zone v, lingual pad with cleat; Zone w, transparent nail varnish; Zones x and y, exposed enamel windows.

Remineralization

Following completion of the demineralization stage, each tooth was gently washed in distilled water, and then placed onto a fresh tube containing either a remineralizing solution (methyl-*p*-hydroxybenzoate, 2.00 g/l; sodium carboxymethyl cellulose, 10.00 g/l; KCl, 8.38 mM; $MgCl_2 \cdot 6H_2O$, 0.29 mM; $CaCl_2 \cdot 2H_2O$, 1.13 mM; K_2HPO_4, 4.62 mM; KH_2PO_4 2.40 mM; fluoride, 0.22 ppm, pH 7.2) for the experimental samples, or distilled water for the control samples. As with the demineralization phase, the teeth were removed at intervals (7, 14, 21 … 49 days), QLF and white light images were taken and the solutions refreshed.

Analysis of lesions

Two main analyses were conducted. The first was a simple clinical visual assessment of the samples each time they were removed from the solution. Following gentle air-drying they were examined by a trained clinician for any sign of demineralization, normally a white spot. If any demineralization was observed the time interval was recorded.

The second analysis was the QLF stage, using the proprietary QLF software (v. 2.00, Inspektor Research Systems, BV, Amsterdam, The Netherlands). A special analysis technique was developed for this study. The analysis patch must surround the lesion, while itself lying on sound enamel. For each lesion, three sides of the patch were placed onto sound enamel (area w) while the final side of the patch lay on the cut medial surface. This portion of the patch was excluded from the analysis, i.e. the software did not consider this fluorescent level as representing sound enamel. This can be seen as a red line on Figure 4c and f. Using a threshold maximum of 40 per cent (i.e. all areas with a percentage fluorescence change from sound enamel of 40 will be removed) the bracket itself was excluded from the lesion analysis. This can be seen in Figure 4c and f. For further details on patch placement and QLF analysis see Pretty *et al.* (2002b). For each tooth the ΔQ value was recorded at a threshold level of 5 per cent, i.e. a minimum of 5 per cent fluorescence loss between sound and demineralized enamel. The examiner followed a defined set of published rules to increase the objectivity of the analysis. All data were entered into SPSS (SPSS Inc, Chicago, USA) for statistical analysis.

Results

Demineralization phase

An example of an experimental tooth following 144 hours of demineralization, with accompanying QLF analysis is shown in Figure 4. The ΔQ for this tooth at 144 hours was 48.3 at the 5 per cent threshold. A control

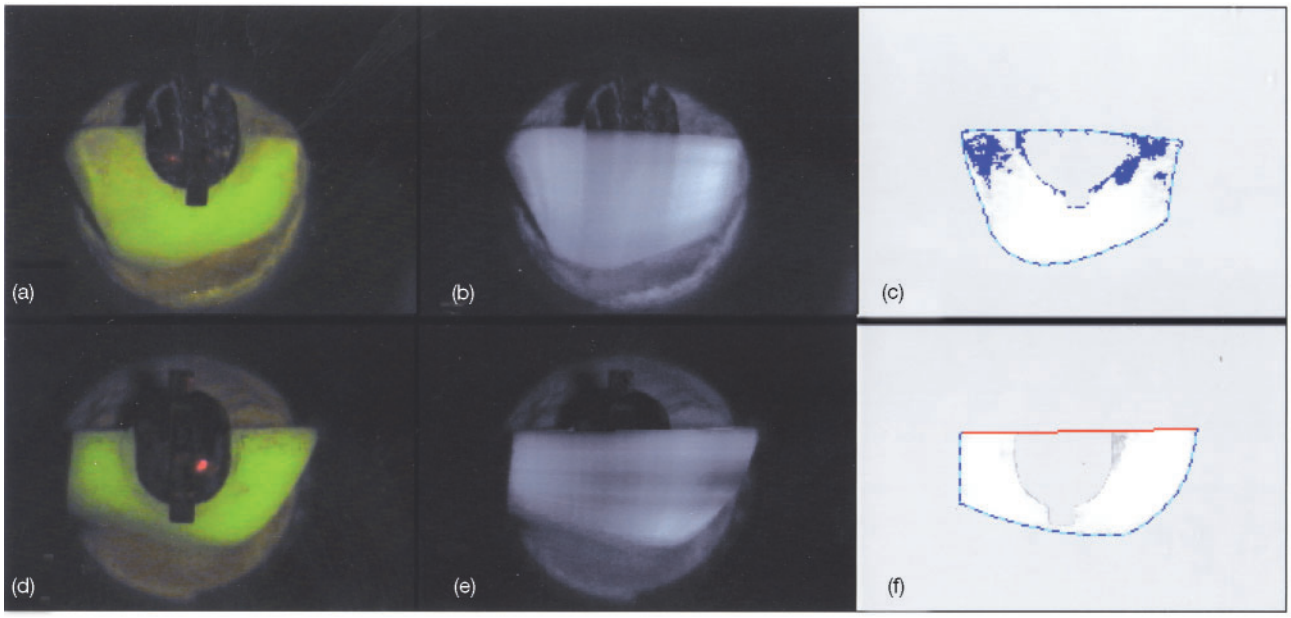

Figure 4 (a) QLF image of a demineralized tooth section, (b) QLF reconstruction of a demineralized tooth, (c) lesion area as quantified by QLF, (d) QLF image of control tooth section, (e) QLF reconstruction of control tooth, (f) QLF analysis demonstrating lack of demineralization.

tooth is also shown, clearly demonstrating the lack of demineralization in the QLF analysis. The ΔQ for this tooth after 144 hours was 0.00 at the 5 per cent threshold. A steady decrease in ΔQ was observed in all the experimental teeth, with a longitudinal increase in fluorescence loss over time. Baseline ΔQ was 0.17 (±0.16), increasing to 5.2 (±1.85) by 24 hours, 29.7 (±9.85) by 144 hours, and by 288 hours it had reached 68.2 (±15.71). Figures 5 and 6 show the individual results for both experimental and control teeth during this phase. All experimental teeth exhibited demineralization with the control teeth remaining sound. Visual examination of the teeth failed to detect any signs of demineralization until 144 hours, and then in only five experimental teeth. At the last examination, visual evidence of demineralization was noted in eight of the 13 teeth. Analysis of the data by ANOVA and subsequent *t*-tests showed statistical differences between the mean ΔQ values for each time period compared with the previous readings (Table 1). No statistical differences were detected by ANOVA between the ΔQ values for the control teeth.

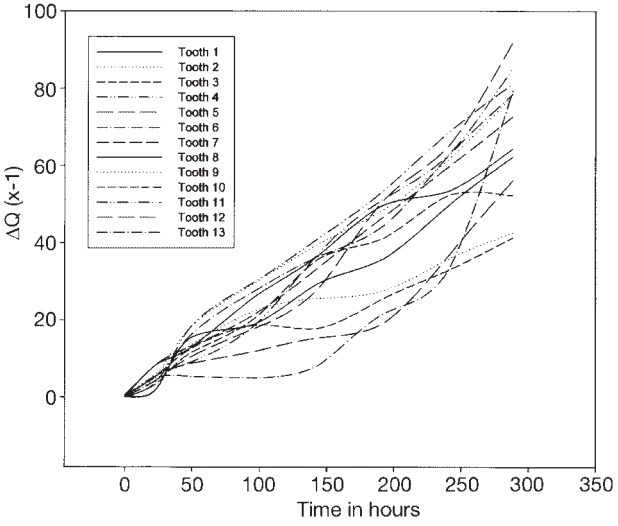

Figure 5 Experimental teeth during the demineralization phase.

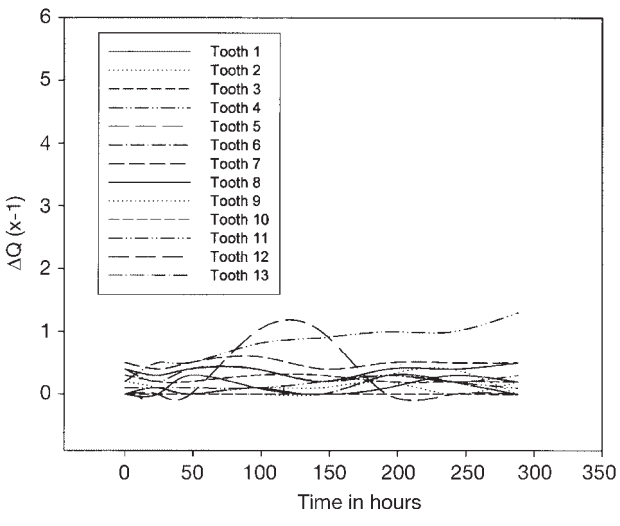

Figure 6 Control teeth during the demineralization phase.

Table 1 Statistical comparisons between measurements of the de- and remineralization phases.

Tooth group	Measure 1 cf. measure 2	Measure 2 cf. measure 3	Measure 3 cf. measure 4	Measure 4 cf. measure 5	Measure 5 cf. measure 6	Measure 6 cf. measure 7	Measure 7 cf. measure 8
Demineralization experimental	$P < 0.001$	$P < 0.001$	$P < 0.000$	$P < 0.000$	$P < 0.000$	$P < 0.000$	$P < 0.000$
Demineralization control	$P < 0.171$	$P < 0.164$	$P < 0.160$	$P < 0.171$	$P < 0.167$	$P < 0.169$	$P < 0.168$
Remineralization experimental	$P < 0.027$	$P < 0.008$	$P < 0.000$	$P < 0.000$	$P < 0.001$	$P < 0.001$	$P < 0.020$
Remineralization control	$P < 0.161$	$P < 0.238$	$P < 0.118$	$P < 0.179$	$P < 0.168$	$P < 0.179$	$P < 0.195$

Remineralization phase

All experimental teeth demonstrated a degree of remineralization, with the control teeth remaining constant. Baseline ΔQ was 68.2 (±15.71), decreasing to 65.5 (±16.2) by 7 days, 50.7 (±15.46) by 28 days, and by 49 days it had reached 38.5 (±14.2) (Figures 7 and 8). Statistical differences between each of the time intervals were detected in the experimental teeth and are shown in Table 1. No significant differences were noted in the control teeth.

The mean values (with standard deviations) for the experimental demineralization and remineralization phases are shown in Figure 9.

Discussion

The demineralization phase of the study demonstrated that not only did QLF detect demineralization, but it was also able to monitor its development longitudinally with increased exposure to the acidic challenge. QLF was also able to detect demineralization before this was visible to the trained examiner. The statistical strength of the differences shows the sensitivity of the technique in detecting these very early lesions.

An aim of early caries detection is that remineralizing therapies can be instituted and thus the risk of aesthetic damage or restorative intervention is avoided (O'Reilly and Featherstone, 1987; McCourt and Cooley, 1991; Donly *et al*., 1995; Hatibovic-Kofman *et al*., 1997). In this study, demineralization occurred beyond that of the first detection, at which point, within a clinical setting, remineralization therapies (normally in the form of fluoride rinses) would be introduced (Øgaard *et al*., 1988b, 1991; Geiger *et al*., 1992; Chadwick, 1994). All the lesions exhibited a degree of remineralization. The remineralization process was longitudinally monitored by QLF detecting highly significant differences over time.

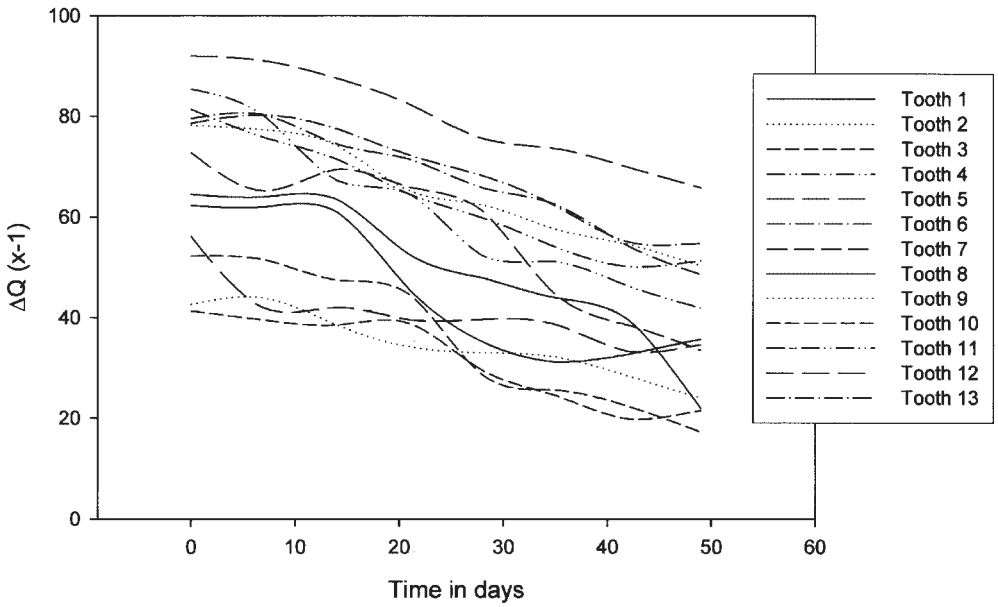

Figure 7 Experimental teeth in remineralizing solution.

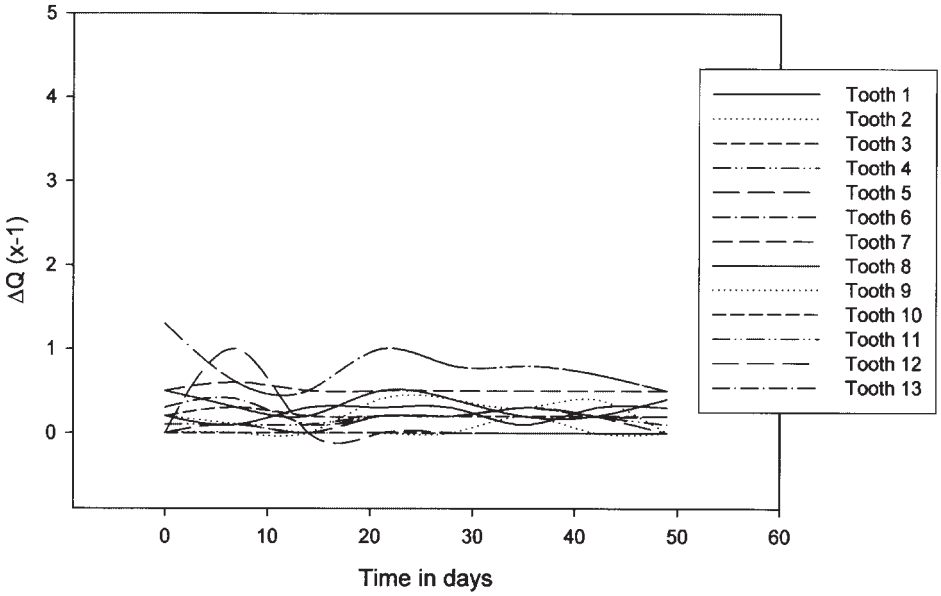

Figure 8 Control teeth in demineralizing solution.

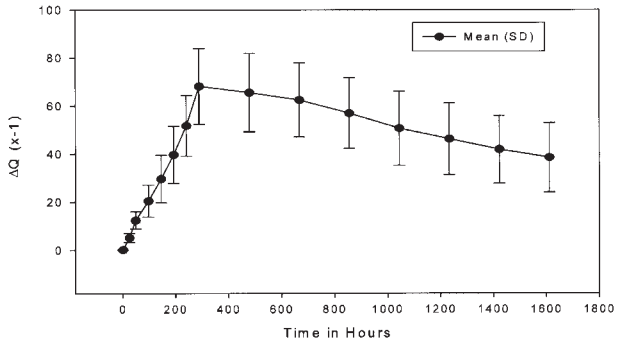

Figure 9 De- and re-mineralization phase (experimental teeth) showing mean values with standard deviations.

The analysis stage of the QLF technique permits the demineralization to be easily identified adjacent to the cleat which, using software thresholds, has been excluded from the analysis (Figure 4). This technique has also been used to detect and monitor demineralization secondary to restorations *in vitro* (Pretty *et al*., 2002a). The reliability of this analysis system has also been proven, demonstrating high levels of intra- and inter-examiner reliability (Pretty *et al*., 2002b).

QLF is an indirect method of demineralization, relying upon the relationship between enamel fluorescence intensity and mineralization status (van der Veen and de Josselin de Jong, 2000). Validation studies have confirmed that this relationship is well correlated (van der Veen and de Josselin de Jong, 2000). Designed as an *in vivo* device, the results of this *in vitro* study suggest that QLF could offer researchers and clinicians alike a new method of detecting and monitoring the demineralization process in orthodontic patients during fixed therapy. Previous work has confirmed the ability to measure such lesions after debonding and thus QLF can be used throughout the entire duration of the treatment (Al-Khateeb *et al*., 1998). The increased interest in reducing risk factors in all, but particularly in elective procedures, ensures that a continued interest in novel methods of demineralization detection will exist.

Conclusions

In this *in vitro* experiment, QLF was able to detect and longitudinally monitor the creation and partial resolution of artificial lesions adjacent to orthodontic cleats on extracted human teeth. Further research is required to ensure that these results can be repeated *in vivo*. QLF represents a potentially useful tool to the orthodontic community.

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