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# Viscoelastic and chemical properties of dentine after different exposure times to sodium hypochlorite, Ethylenediaminetetraacetic acid and calcium hydroxide


## Abstract

This study aims to evaluate the viscoelastic and chemical properties of dentine after different durations of exposure to 5.25% NaOCl, 17% EDTA and $Ca(OH)_2$ solutions, and NaOCl in alternating combination with EDTA. Standard dentine bars were randomly assigned to: (1) formal-saline control-1; (2) NaOCl; (3) EDTA; (4) NaOCl/EDTA; (5) formal-saline control-2; (6) $Ca(OH)_2$ pH 12.6; (7) $Ca(OH)_2$ pH 9.8. Groups 1-4 underwent 10-min cycles of soaking and Dynamic Mechanical Analysis up to 120 min. Groups 5–7 underwent similar tests at days 7, 14, 28, 84. FTIR spectra of dentine discs exposed to the same regimens assessed surface chemistry. NaOCl, or $Ca(OH)_2$ (pH12.6) solutions reduced the organic (N-H[1], N-H[3], C=0) peak components of dentine. This study demonstrated that accumulative damage of dentine could be facilitated by alternated exposure to NaOCl and EDTA. Exposure of dentine to $Ca(OH)_2$ (pH12.6) for 7 days reduced viscous behaviour, inferring increased potential for fatigue failure.




**Key words:** calcium hydroxide, dentine, EDTA, sodium hypochlorite, viscoelastic

## Introduction

Mechanically, teeth are non-repairing, complex hard tissues, designed to sustain masticatory cyclic loading over elongated life-spans, although they may succumb abruptly to traumatic impact injuries (1). The reputedly higher fracture susceptibility of *root-treated* teeth may be due to lost tooth structure, altered proprioception or mechanical behaviour of dentine. The latter has been attributed to loss of pulp vitality and exposure of roots to various chemicals (2). Repeated 30-minute irrigation steps with 5.25% NaOCl in teeth increased cervical tooth surface strain that plateaued after two steps (3), whereas, alternate irrigation with 5% NaOCl/17% EDTA in 30-minute cycles eliminated the plateau (4), allowing continued increase. The altered mechanical behaviour is likely due to depletion of its organic component by NaOCl (5) and the mineral component by EDTA (6). The effect of subsequent calcium hydroxide dressing for different durations on fracture strength has been confirmed in immature sheep (7) and human (8) incisors. Tests on dentine bars immersed in solutions of $Ca(OH)_2$, found the elastic modulus to be unaltered but flexural strength to be significantly decreased (9, 10).

Whole tooth deformation is complex and explained by the relative deformation of bone, periodontal ligament and tooth structures. The strain behaviour of “whole teeth” is complex (11) and not fully explained by quasi-static properties (flexural strength, elastic modulus) of small dentine samples, which are significantly affected by soaking in NaOCl, EDTA and $Ca(OH)_2$ (3, 9). Proportional limit, ultimate tensile and micro-punch shear strength of dentine have also been investigated but much still remains to be understood (12, 13). The time-dependent mechanical behaviour of dentine samples at low cyclic loads may offer better explanation of the behaviour of whole teeth loaded cyclically within their elastic limit (4). However, the effect of root canal irrigants and dressings on the viscoelastic properties of dentine has not been fully tested. Also, the effect of prolonged exposure of dentine to irrigants requires further exploration because it is common for clinicians to flood the pulp

chamber and root canals with NaOCl during the entire period of chemo-mechanical preparation (14).

Given the likely importance of viscoelastic properties of teeth on their survival, the aim of this study was to evaluate the time-dependent effect of NaOCl (5.25%) and EDTA (17%), independently, or in combination, as well as the effect of $Ca(OH)_2$ (pH 12.6 or 9.8) on the viscoelastic behaviour of dentine using DMA; and explain the changes using FTIR. The null hypotheses for the study were that there was no significant effect of the exposure time of NaOCl (5.25%), EDTA (17%) or $Ca(OH)_2$ (pH 12.6 or 9.8) solution on the storage modulus ($E'$) and tangent delta (tan δ) of dentine, with significance set at the 5% level.

## Materials and Methods

### *Preparation of dentine specimens*

Human extracted teeth for this study (Study ID: 1301) were supplied by the UCL Eastman Biobank having received favourable opinion from Yorkshire & The Humber – Leeds East Research Ethics Committee (REC reference: 17/YH/0100). Disease-free, recently extracted, intact ~~wisdom teeth~~third molars were donated with informed consent by treated patients (age 18 – 34 years) in the Oral Surgery Department (Eastman Dental Hospital) and stored in 4% formal-saline (BDH Laboratory Supplies, Poole, UK) for no longer than four weeks prior to experimentation. Adherent hard and soft tissue was removed and the teeth decoronated at the cemento-enamel junction. Longitudinal plano-parallel bars were cut from the long axis of the roots, parallel to the bucco-lingual surfaces, using a diamond-coated saw under constant water coolant (Model 60, South Bay Technology inc., Basingstoke, UK). The plane of longitudinal section was at right-angles to the tubule direction.

One hundred and five dentine bars (105 teeth) of standard dimensions (12 mm × 0.8 mm × 2 mm) were produced; each measured for any variation with a micrometer (Mitutoyo, Kawasaki, Japan) and randomly assigned to one of seven groups for DMA testing (Table 1).

In addition, 21 square dentine discs (4 mm × 4 mm × 1 mm) were prepared from the crowns of 21 teeth and randomly subjected to the designated treatments for FTIR evaluation.

***Preparation of test solutions***

Sodium hypochlorite (12.75% - BDH Laboratory Supplies, Poole, UK) was diluted with distilled water and iodometrically titrated to obtain the available chlorine concentration of 5.25%, stored in opaque polyethylene containers and used within 48 h. EDTA (17%) solution was obtained by dissolving EDTA salt into distilled water with sodium hydroxide salt on a slow magnetic stirrer to pH 7.8. A saturated solution of calcium hydroxide was prepared by dissolving 40 g of $Ca(OH)_2$ (BDH Laboratory Supplies, Poole, UK) overnight in 1 L of distilled water and filtered under vacuum (Whatman, Maidstone, UK). The harvested supernatant of pH 12.6 was diluted to give a second solution of pH 9.8.

***Treatment of the dentine bars and discs***

The 60 dentine bars (n = 15 per group) and 12 square discs (n = 3 per group) from groups 1-4 were immersed in their respective test solutions (25 mL) (Table 1) for 10 minutes in each cycle of soaking and loading for up to 120 minutes. The tests for groups 1–4 were conducted at baseline and repeated 12 times for each sample. The test solutions were freshly changed after each 10-minute soak-period. The group 4 dentine bars were initially soaked in 5% NaOCl for 10 minutes, rinsed in distilled water for 10 minutes, and tested by Dynamic Mechanical Analysis (DMA) ~~tested~~; the bars were then soaked in 17% EDTA for 10 minutes, rinsed in distilled water, and DMA-tested again. These two cycles were conducted 6 times. Each bar was marked for orientation to reproducibly return them to the DMA supports in retests.

The 45 bars (n = 15 per group) and 9 square discs (n = 3 per group) from groups 5–7 were individually immersed in the respective test solutions (25 mL) for 84 days (Table 1). They were DMA-tested at days 0, 7, 14, 28 and 84. Each test was preceded by rinsing in 25 mL distilled water for 10 minutes, blotting on absorbent tissue and air-drying for 10 minutes to

achieve constant weight. The pre-DMA-test weight of each "air-dried" dentine bar was established using an electronic balance (BDH, Poole, UK), and repeated after the DMA-test.

***Dynamic Mechanical Analysis (DMA) conditions***

Each dentine bar was evaluated using flexural testing in a Perkin-Elmer DMA-7, operated with the Pyris software (Perkin-Elmer Corp USA). A 3-point bend testing accessory was used with a 10 mm span length yielding an average aspect ratio of approximately 13 with a tolerance of +4 or -2 (American Society for Testing and Materials D5023-95a). The loading was parallel to the dentinal tubules. Testing was carried out where the static stress (240-360 mN) was maintained at a ratio of 1.2 relative to the dynamic stress (200-300 mN), which was applied at a frequency of 1 Hz. A dynamic strain control was maintained at 0.02%. Testing was performed at room temperature for 1 minute and data recorded at 30 s. DMA data were presented as storage modulus ($E'$) and tangent delta (tan δ) *versus* soaking time.

***Fourier Transform Infra-red Spectrometry (FTIR) tests***

Infra-red spectroscopy was performed on treated discs to evaluate compositional change (Perkin Elmer series 2000 FTIR spectrometer; Perkin-Elmer Corp, Waltham, Massachusetts, USA). The absorbance range was set between 500 $cm^{-1}$ and 4000 $cm^{-1}$; the resolution was set at 8; and the number of scans was 4. The average spectrum of each set of four repeated scans, and absorbance for each averaged spectrum at specific wavenumbers were obtained using Spectrum® software (Perkin Elmer series 2000). FTIR peaks were assigned for the main components of dentine and compared with existing data (15, 16). The collagen and phosphate peak heights at 1640 $cm^{-1}$ and 1000 $cm^{-1}$ were obtained by subtracting background absorbance at 1730 $cm^{-1}$ and 1180 $cm^{-1}$, respectively.

***Statistical analysis***

$E'$ and Tan δ values were calculated using Pyris software; each dentine bar had 13 (groups 1–4) or 5 (groups 5–7) measurements. Kolmogorov-Smirnov and Shapiro-Wilk tests for Normality revealed the data fulfilled the assumption of normal distribution. Mean and

standard deviation values for $E^I$ and tan δ were calculated for each group. Repeated Measures ANOVA with Bonferroni pairwise comparisons were performed to investigate the effect of exposure time on $E^I$ and tan δ, (STATA 12; STATA Corporation: College Station, TX, USA), at a significance level of 5%.

## Results

***Groups 1–4 (exposure to 5.25% NaOCl and/or 17% EDTA solutions)***

The mean $E^I$ values plotted against time for each group are shown in Figure 1. Immersion in 17% EDTA (Group 3) resulted in a small (Range ~ 10-13 GN/m$^2$) but statistically significant decrease in $E^I$ with time ($P$ = 0.0008). Significant decrease in $E^I$ values (elastic properties) from the baseline was detected after immersion for 60 minutes ($P$ < 0.0001) with no further significant change, thereafter. This time-dependent decrease was more marked (Range ~ 10-14 GN/m$^2$) and statistically significant when samples were immersed alternately in 5.25% NaOCl and 17% EDTA (Group 4) ($P$ = 0.002); the significant change was evident after the 4$^{th}$ cycle of immersion (40 minutes in NaOCl plus 40 minutes in EDTA) ($P$ < 0.0001) with no significant change, thereafter.

The mean tan δ values plotted against time for each group are also shown in Figure 1. Immersion in 5.25% NaOCl (Groups 2) significantly increased tan δ (viscous behaviour) ($P$ = 0.004); the change was noted after the first 10-minute of immersion with no further increase thereafter. Immersion in 17% EDTA (Group 3) ($P$ = 0.002) resulted in a more consistent increase in tan δ over time but significant change was only detected after 30-minute exposure, with no significant increase thereafter. A significant increase in tan δ was also observed when dentine bars had been immersed alternately in 5.25% NaOCl and 17% EDTA (Group 4) ($P$ < 0.0001). The increase was significant after 30-minute exposure and gradually continued upto to 70 minutes when a significant ($P$ = 0.02) decrease was noted at 80 minutes with no obvious change, thereafter.

Average FTIR spectra (normalised by the hydroxyapatite [HA] peak at 1000 $cm^{-1}$) of unreacted samples and those reacted with 5.25% NaOCl are shown in Figure 3. The effect of exposure of dentine to 17% EDTA is shown in the spectra normalised by N-H(3) at 1235 $cm^{-1}$ (Figure 4). Spectra of dentine discs reacted alternately to 5.25% NaOCl and 17% EDTA revealed considerable reduction of the hydroxyapatite peak (HA) at 1000 $cm^{-1}$ and an appreciable decrease in the organic components: N-H(1) and N-H(2) bands, which mirrored the FTIR spectra for samples reacted to 5.25% NaOCl alone (Figure 5). The water peak (3200 $cm^{-1}$) had also increased substantially.

***Groups 5–7 (exposure to calcium hydroxide solutions)***

The plotted mean data for each group showed consistent but small changes in individual specimens over the twelve-week period (Figure 2). One dentine bar in Group 7 fractured on day 0, reducing the sample size to 14. There was significant decrease in $E'$ (elastic properties) of dentine bars in group 5 (formal-saline control-2) ($P = 0.0002$) and Group 7 (pH 9.8) ($P = 0.02$) after 84 days exposure. There was also a small but statistically significant reduction in tan δ (viscous behaviour) of the dentine bars over the duration of exposure to pH 12.6 solution (group 6) ($P < 0.0001$); the significant change was noted after 7 days ($P = 0.001$).

FTIR spectra (normalised at 1000 $cm^{-1}$) showed that the “C=O” and “N-H” peaks at 1640 $cm^{-1}$ and 1540 $cm^{-1}$, respectively, in the organic part of the spectrum, progressively diminished with reaction time with $Ca(OH)_2$, regardless of pH (Figure 6).

## Discussion

The null hypotheses were rejected as significant changes in the viscoelastic properties of dentine were found upon controlled soaking in NaOCl, EDTA and $Ca(OH)_2$ using Dynamic Mechanical Analysis (DMA). DMA ~~is used to~~ characterises the viscoelastic properties of materials~~,~~ employing loads within the elastic limit, without permanent sample deformation. It ~~DMA~~ measures the amplitudes of stress and strain, as well as the phase angle between

them, enabling resolution of modulus into in-phase (storage modulus; $E^{I}$ representing elastic behaviour) and out-of-phase (loss modulus; $E^{II}$ describing viscous behaviour) components (12). The relative amount of energy lost to that stored, indicates damping (tan $\delta$ = loss modulus $E^{II}$ / storage modulus $E^{I}$).

The proportions of mineral, organic and water (17) dictate the mechanical behaviour of mineralised tissues. The mineral (hydroxyapatite) provides the strength and elasticity, collagen the toughness, and hydrated (plasticised) collagen, the viscous element. Dentine is a composite-fibre structure, peritubular dentine acting as a hypermineralised "fibre" within the intertubular matrix. Tubule direction and the perpendicularly-oriented collagen fibre matrix may contribute to anisotropy (12), which was not evaluated in this study. The close conjunction between mineral and collagen, by virtue of intra-fibrillar and extra-fibrillar collagen mineralisation provides a pre-stressed composite, which upon dehydration consolidates the granular mineral matrix by shrinkage of the interpenetrating collagen, thereby increasing Young's modulus.

The extracted tooth samples were stored in 4% wt formal-saline for cross-infection control because it does not alter either the FTIR spectra (6) or their mechanical properties (18). Prolonged storage in 4% formal-saline for upto 84 days (group 5 – control 2) significantly reduced $E^{I}$ (elastic behaviour) to a small extent, consistent with loss of mineral (19).

A two-hour test span for irrigants was chosen for comparability with previous work (3, 9) and clinical relevance (14). The duration of dentine exposure to EDTA during treatment varies between clinicians and the specific rationale for its use. Some advocate EDTA as a routine penultimate root canal irrigation step (20), others as a chelant to aid bacterial load reduction (Bystrom & Sundqvist 1985) and biofilm degradation (2, 21), to remove packed debris from accessory anatomy (22) and open up calcified canal anatomy, where the duration may extend to 2 h (14). The experimentation partitioned the two-hour test span at 10-minunte intervals for a more precise time-dependent assessment of change in viscoelastic behaviour (23).

Calcium hydroxide dissociates into calcium and hydroxyl ions in an aqueous solution with a low solubility product, meaning that it is only partially solution, which is important to effect a lasting effect as depletion of the hydroxyl ion drives the equation equilibrium towards more dissolution, thereby maintaining the ionic concentration. The tissue dissolution and antibacterial actions of $Ca(OH)_2$ are attributed to the effect of the hydroxyl ion. It was considered important to control this factor so that the concentration and pH were maintained. Use of a commercial product would entail the inclusion of other additives that may interfere with this control, hence a saturated solution calcium hydroxide was used.

The test specimens were washed and “air dried” for 10 minutes before testing because it was not possible to test soaking-wet samples. Dehydration affects dentine properties but is dependent on the level of dehydration. The “drying” protocol was tested in pilot experiments at 10-minute intervals over a 2-hour period; measurable change in elasticity was only detected after sixteen minutes of dehydration. The extent of drying in this study therefore had a negligible impact on the results.

In the present study, a constant dynamic strain control of 0.02% at 37°C was not achievable; all tests were therefore run at room temperature. The approach would mitigate dentine dehydration that could occur at higher temperatures and should not alter the final effect but only the rate of reaction. The loading frequency of 1 Hz was consistent with masticatory frequency (1-2 Hz) (1); this combined with the 1-minute test period decreased the potential of unwanted strain in the test samples.

The relative lack of effect on $E'$ by 5.25% NaOCl seems in marked contrast to the changes reported for quasi-static properties of dentine bars, where Young’s modulus and flexural strength were reduced significantly (3, 9). The data are consistent with the limited tooth surface strain increase (3) evident when irrigating whole teeth. The elastic properties remained unaltered, presumably due to the relative integrity of the mineral phase through its volume (6). It is likely that the minimal but early increase in tan δ was due to surface collagen degradation limited by the hydroxyapatite (6).

The dentine bars in the EDTA group exhibited appreciable and significant increases in tan δ, coupled with reduction in $E^l$. EDTA removes the mineral component (Figure 4), reducing elastic properties. The limited changes in the first 30-minute of exposure may be due to progressive removal of any smear layer (also penetrating dentinal tubules) and subsequently limited by the remaining intact collagen (6). The smear-free tubules and loss of hydroxyapatite from the peritubular and intertubular dentine may allow greater hydration of exposed collagen, explaining the increase in tan δ. However, any further continuing effect may have been limited by re-deposition of mineral or limited penetration along the dentinal tubules.

The dentine bars exposed alternately to 5.25% NaOCl and 17% EDTA, exhibited significant increases in tan δ (after 30 minutes) and marked decrease in $E^l$ (after 80 minutes), consistent with the above observations. The altered mechanical behaviour is also consistent with the increase in tooth surface strain following the same irrigation regimen (4). The significant increase in viscous deformation of dentine, during the first 70 minutes of alternate exposure infers deeper chemical effects on both mineral and collagen phases by the respective agents (6). The increased viscous behaviour infers increased capacity for energy dissipation under stress, coupled with reduced risk of brittle fracture within the elastic limit; however, the simultaneous reduction in modulus would be consistent with reduced dentine fracture toughness after alternate exposure to NaOCl and EDTA.

The discontinuity in data represented by the abrupt change in tan δ and $E^l$ (although less obvious in the latter) between 70 and 80 minute of NaOCl / EDTA treatment was consistent in 13 of 15 samples. This discontinuity was also evident in groups 1, 2, 3, suggesting cumulative mechanical fatigue, bearing in mind the parallel tubule orientation to loading direction. It is speculated that creep in dentine matrices (24) may slowly alter local stress concentrations at the points of contact between the sample and supports on the test platform, as well as the loading probe. The heightened effect in group 4, may reflect greater instability at the points of contact. FTIR revealed substantial reduction in the HA and N-H(2) peaks from 50- to 80-minute of exposure and explain the mechanical findings. The effects

seen in the EDTA/NaOCl group may potentially reflect an increased susceptibility to crack propagation without the protective mechanisms of hydrated collagen (25).

Dentine bars exposed to stock (group 6) or diluted (group 7) calcium hydroxide solutions only exhibited small changes in their $E^l$ after immersion for 84 days in diluted solution. This seems consistent with the micromechanical model because a saturated calcium solution, albeit at a high pH, may deplete the mineral content (Figure 6) of dentine to a small extent (19). The bars exposed to the stock solution (pH 12.6) (group 6) exhibited reduced viscous behaviour, possibly due to complete denaturation of the exposed collagen (26), which may in turn influence its state of hydration and thus viscoelasticity (27). The calcium hydroxide solutions exhibited constant initial pH, which dropped upon exposure to dentine, explained by depletion of hydroxyl ions through reaction with dentine collagen (28). The findings are consistent with the observation that dentine bars exposed to calcium hydroxide did not exhibit altered Young's modulus (9, 10) but reduced flexural strength (7, 8). Grigoratos *et al.* (9) explained the disparate effects by the observation that the restricted surface effect would influence the two properties differently. Further research to explore the location of boundary of such chemical and physical changes is merited.

The present findings emphasise the need for caution in selecting the duration of alternated exposure to 17% EDTA and 5.25% NaOCl solutions (4, 29), although greater frequency of alternation between the two solutions clinically may simply neutralise NaOCl (30) and hence the overall effect on tooth surface strain (23). Neutralisation was avoided in the present study by washing with saline between alternate exposures to each solution, allowing the accumulative effect to manifest.

## Conclusions

The present data signify that NaOCl and EDTA independently may influence dentine viscoelasticity in different and self-limiting ways over the duration of exposure. However, alternation between EDTA and NaOCl facilitates accumulative damage. $Ca(OH)_2$ at pH 12.6

induced significant reduction in viscous behaviour (tan δ) of dentine, inferring increased potential for fatigue failure.

## Figure captions

**Figure 1** The mean and standard deviation values for storage modulus (EI) (GNm-2) and tan delta (tan δ) plotted against time for groups 1–4 (Group 1 Formal-saline; Group 2 NaOCl; Group 3 EDTA; Group 4 NaOCl/EDTA).

**Figure 2** The mean and standard deviation values for Storage Modulus ($E^{l}$) ($GNm^{-2}$) and Tan delta (tan δ) plotted against time for groups 5–7 (Group 5 Saline; Group 6 $Ca[OH]_2$ pH 12.6; Group 7 $Ca[OH]_2$ pH 9.8).

**Figure 3** Average FTIR spectra (n = 3) of dentine discs reacted with 5.25 wt% NaOCl for 0, 20, 50, 80 or 110 minutes.

**Figure 4** Average FTIR spectra (n = 3) of dentine discs reacted with 17 wt% EDTA for 0, 20, 50, 80 or 110 minutes.

**Figure 5** Average FTIR spectra (n = 3) of dentine discs reacted with 17 wt% EDTA / 5.25 wt% NaOCl for 20, 50, 80 or 110 minutes.

**Figure 6** Average FTIR spectra of dentine discs reacted with $Ca(OH)_2$ at pH 12.7 (high) or 9.8 (low) for 28 days.

## Table caption

**Table 1** Group composition, test conditions and sample size.

**Table 1: Group composition, test conditions and sample size**

| Group | Test Media | No. of dentine bars for DMA | Soaking cycle | Washing cycle in $H_2O$ (10 mins in 25 mL) | Test cycle (1 min) | Cycle repeats | Data points |
|---|---|---|---|---|---|---|---|
| **Group 1 (Control-1)** | 4% Formal-saline | 15 | 10 min in 25 mL | ✓ | ✓ | 12 | 13 |
| **Group 2** | 5.25% NaOCl | 15 | 10 min in 25 mL | ✓ | ✓ | 12 | 13 |
| **Group 3** | 17% EDTA | 15 | 10 min in 25 mL | ✓ | ✓ | 12 | 13 |
| **Group 4** | 17% EDTA / 5.25% NaOCl | 15 | 10 min in 25 mL | ✓ | ✓ | 12 | 13 |
| **Group 5 (Control-2)** | 4% Saline | 15 | 1, 2, 4, 12 wks in 25 mL | ✓ | ✓ | 4 | 5 |
| **Group 6** | $Ca(OH)_2$ with high pH (12.7) | 15 | 1, 2, 4, 12 wks in 25 mL | ✓ | ✓ | 4 | 5 |
| **Group 7** | $Ca(OH)_2$ with low pH (9.8) | 15 | 1, 2, 4, 12 wks in 25 mL | ✓ | ✓ | 4 | 5 |
| **Total** | | **105** | | | | | |

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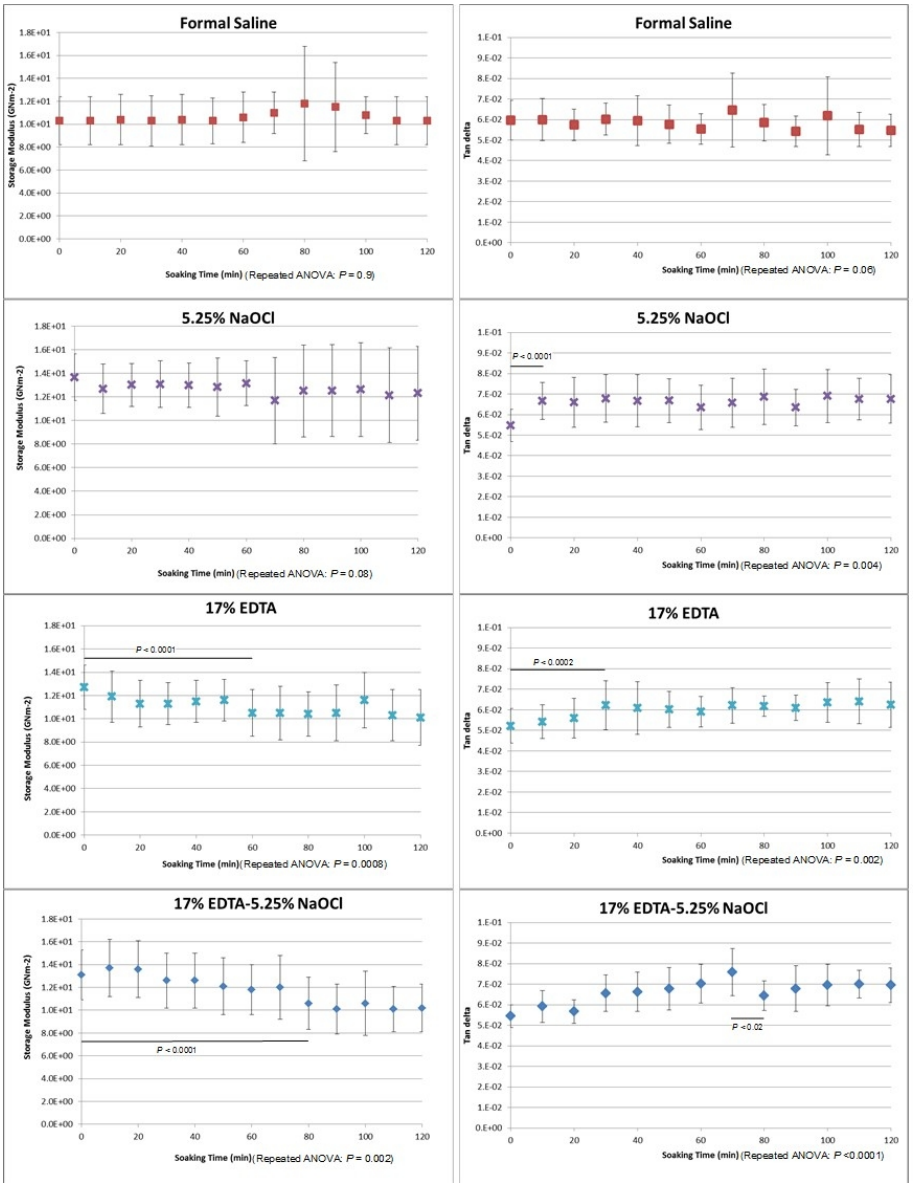


Figure 1 shows the mean and standard deviation values for storage modulus (EI) ($GNm^{-2}$) and tan delta (tan δ) plotted against time for groups 1–4 (Group 1 Formal-saline; Group 2 NaOCl; Group 3 EDTA; Group 4 NaOCl/EDTA).

161x190mm (150 x 150 DPI)

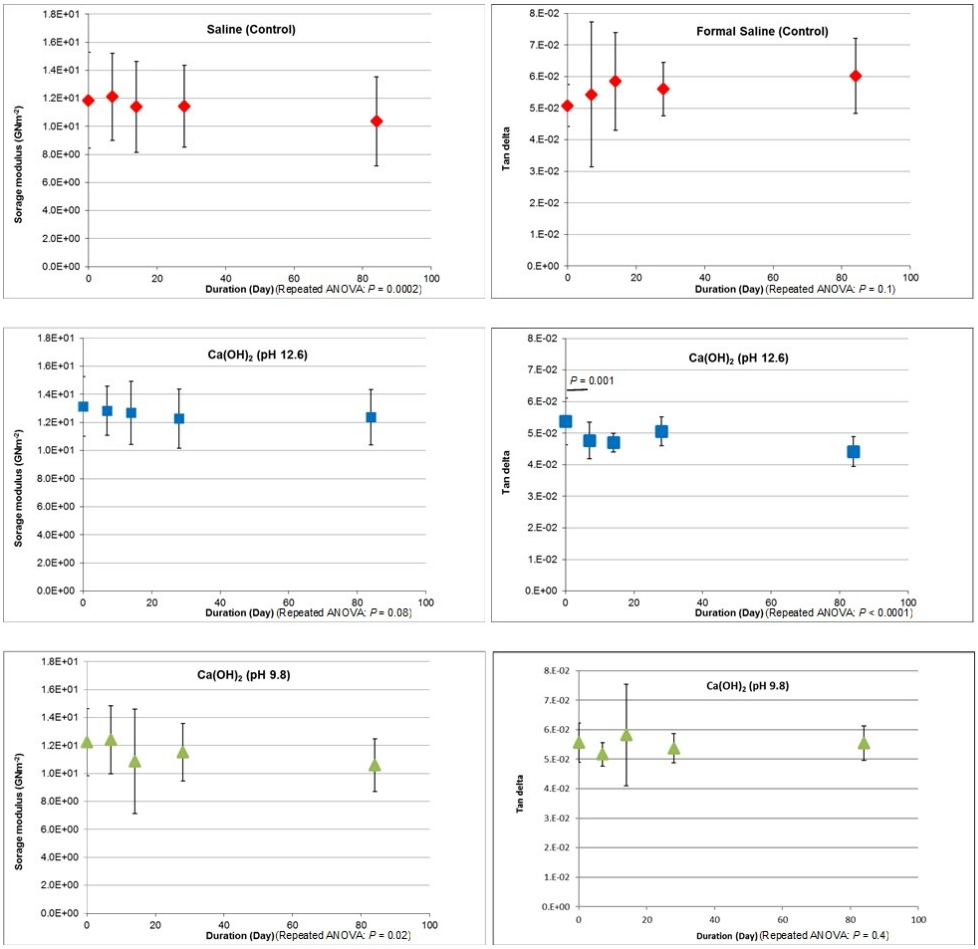


Figure 2 shows the mean and standard deviation values for Storage Modulus (EI) ($GNm^{-2}$) and Tan delta (tan δ) plotted against time for groups 5–7 (Group 5 Formal-Saline; Group 6 $Ca[OH]_2$ pH 12.6; Group 7 $Ca[OH]_2$ pH 9.8).

170x160mm (150 x 150 DPI)

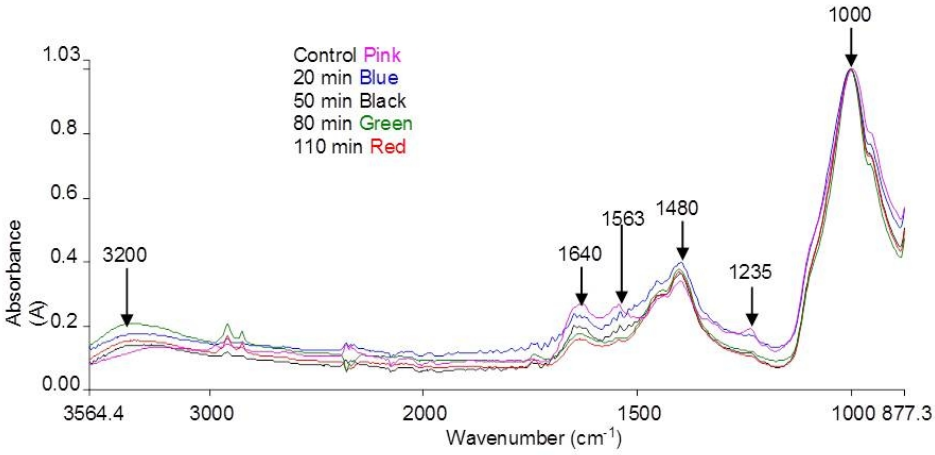


Figure 3 Average FTIR spectra (n = 3) of dentine discs reacted with 5.25 wt% NaOCl for 0, 20, 50, 80 or 110 minutes.

159x96mm (150 x 150 DPI)

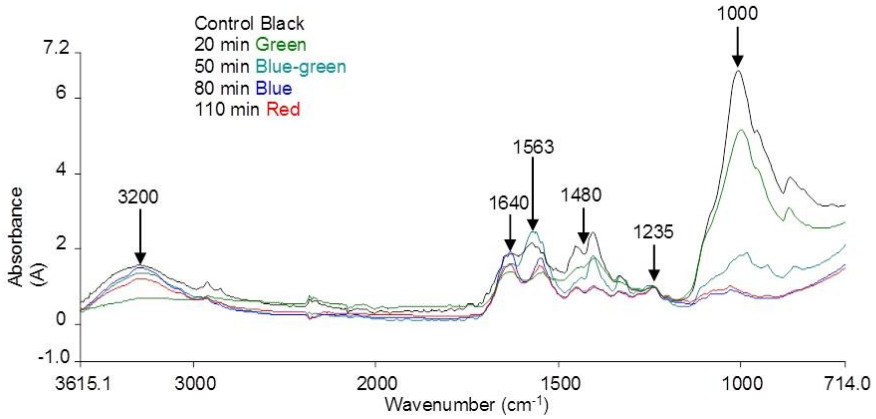


Figure 4 Average FTIR spectra (n = 3) of dentine discs reacted with 17 wt% EDTA for 0, 20, 50, 80 or 110 minutes.

170x77mm (150 x 150 DPI)

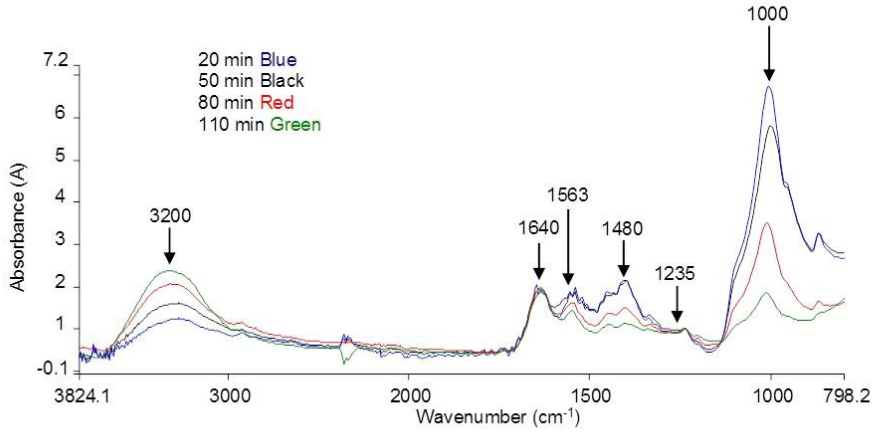


Figure 5 Average FTIR spectra (n = 3) of dentine discs reacted with 17 wt% EDTA / 5.25 wt% NaOCl for 20, 50, 80 or 110 minutes.

170x100mm (150 x 150 DPI)

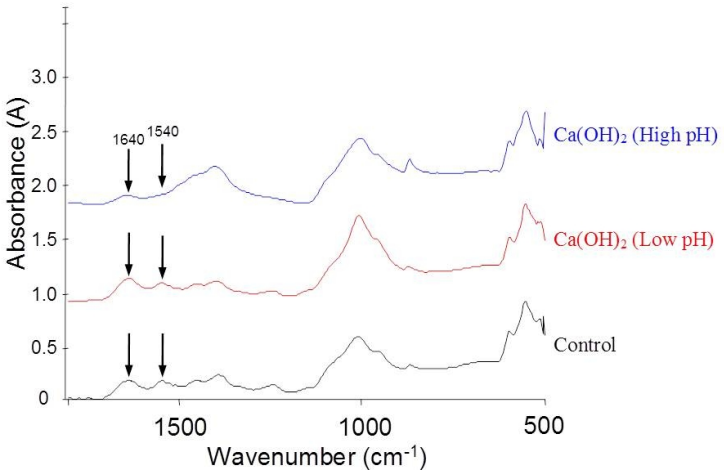


Figure 6 Average FTIR spectra of dentine discs reacted with $Ca(OH)_2$ at pH 12.7 (high) or 9.8 (low) for 28 days.

207x125mm (150 x 150 DPI)