Effect of root canal irrigant (sodium hypochlorite & saline) delivery at different temperatures and durations on pre-load and cyclic-loading surface-strain of anatomically different premolars

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Running title: Strain limits of teeth under chemical, thermal and mechanical stresses

Key words: Tooth surface strain, sodium hypochlorite, saline, irrigant temperature, pre-load strain, cyclic loading strain

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Abstract

Aim: To evaluate the effect of NaOCl (5%) and saline (control) irrigant delivery at different temperatures and durations on pre-load and cyclic-loading tooth-surface-strain (TSS) on anatomically different premolars.

Methodology: Single-rooted premolars (n=36), root-canal-prepared in standard manner, were randomly allocated to six irrigation groups: (A1) NaOCl-21°C; (A2) NaOCl-60°C; (A3) saline-21°C then NaOCl-21°C; (A4) saline-60°C then NaOCl-21°C; (A5) saline-21°C then NaOCl-60°C; (A6) saline-60°C then NaOCl-60°C. A1-2 received nine 10-min irrigation periods (IP) with NaOCl; A3-6 received nine 10-minute IP with saline, followed by 9 IP with NaOCl at different temperature combinations. Premolars (n=56) with single, fused or double roots prepared by standard protocol, were stratified and randomly allocated to: (B1) saline-21°C; (B2) saline-80°C; (B3) NaOCl-21°C; (B4) NaOCl-80°C. TSS (µє) was recorded pre-irrigation, post-irrigation and pre-load for each IP and during cyclic loading 2 min after each IP, over 30-274 min, using strain-gauges. Generalised linear mixed models were used for analysis.

Results: Baseline TSS in double-rooted premolars was significantly (*p=0.001*) lower than in single/fused-rooted-premolars; and affected by mesial-wall-thickness (*p=0.005*). There was significant increase in loading-TSS (µє) after NaOCl-21°C irrigation (*p=0.01*) but decrease after NaOCl-60°C irrigation (*p=0.001*). TSS also increased significantly (*p=0.005*) after Saline-80°C irrigation. *Pre-load* "strain-shift" was noted only upon first saline delivery but every-time with NaOCl. Strain-shift negatively influenced loading-TSS after saline or NaOCl irrigation (A3-6) but was only significant for saline-21°C.

Conclusions: Tooth anatomy significantly affected its strain characteristics, exhibiting limits within which strain changes occurred. Intra-canal introduction of saline or NaOCl caused non-random strain shifts without loading. Irrigation with NaOCl-21°C increased loading tooth strain, as did saline-80°C or NaOCl-80°C but NaOCl-60°C decreased it. A "chain-link" model was proposed to explain the findings and tooth biomechanics.

INTRODUCTION

Teeth appear to be simple structures on a gross scale but display astounding beauty and complexity at micro and nanoscopic scales that reflects their functional and mechanical behaviour (Currey 1999). The “pre-stressed laminate” behaviour of intact teeth was hypothesised long before proof of mechanisms emerged (Tidmarsh 1976). Such “pre-stress” maybe due to the dynamic interaction between collagen molecules, nanometre-sized mineral particles and the water content in dentine (Bertinetti *et al.* 2015, Forien *et al.* 2016). These interactions provide strengthening and toughening mechanisms designed to prevent crack propagation in a non-repairing hydrated mineral tissue (Zaslansky *et al.* 2016). The response to tooth-loading is also moderated by tooth architecture and its periodontal support structure (Naveh *et al.* 2012, Nikolaus *et al.* 2017). Despite the evolving insight about tooth biomechanics, much still remains to be discovered about their fatigue strength and long-term behaviour.

Disease and restorative procedures weaken teeth by disrupting their architecture and ability to respond favourably to loading (Vukicevic *et al.* 2015). Root canal treatment is a procedure used to control root canal infection and periapical disease by irrigation of its fine capillary-like canals using sodium hypochlorite (Gulabivala *et al.* 2019). Root canal treatment may incur further tooth structure disruption through canal preparation (Lang *et al*. 2006) and chemical (Sim *et al*. 2001, Grigoratos *et al*. 2001, Rajasingham *et al.* 2010) or thermal (Karunanayake *et al.* 2018, Kafantari *et al.* 2018) damage by alteration of compositional (O’Driscoll *et al.* 2002, Pascon *et al.* 2012, Ramirez-Bommer *et al*. 2017) and mechanical properties (Sim *et al*. 2001, Grigoratos *et al*. 2001, Rajasingham *et al.* 2010).

The commonly adopted sodium hypochlorite (NaOCl) irrigant for root canal treatment causes chemical damage to dentine (O’Driscoll *et al.* 2002). Since both antimicrobial (Cunningham & Joseph 1980, Sirtes *et al.* 2005) and tissue-dissolving (Cunningham & Balekjian 1980, Abou-Rass & Oglesby 1981) properties are enhanced by higher temperature, *heated NaOCl* has been advocated for enhancing root canal debridement potential during clinical use (Ruddle 1994, Castelluci 2004). Heating devices for endodontic irrigating syringes (EndoWarmer® [Albrina, Forli, Italy], Syringe-

Warming Device [Keydent, Vaterstetten, Germany]) allow NaOCl to be heated to as high as 80°C before introduction into the root canal system or alternatively other strategies involve intra-canal heating of solutions (de Hemptinne *et al.* 2017, Leonardi *et al*. 2019). Although, the effect of heat and *heated* NaOCl on the mechanical properties of dentine bars has been determined (Karunanayake *et al.* 2018, Kafantari *et al.* 2018), its effect on whole teeth (Sim *et al*. 2001) during root canal irrigation has not.

Heat may induce evaporation of unbound dentine water as well as loss of bound water at temperatures above 200°C (Helfer *et al*. 1972). Since water is integral to the viscoelastic properties of dentine (Craig & Peyton 1958), its loss would compromise the mechanical properties of teeth (Huang *et al.* 1992, Jameson *et al.* 1993, Kishen *et al.* 2001).

Heat may also compromise dentine properties directly by altering intra-dentinal collagen properties. Collagen consisting of 3 helical polypeptide chains bound together by hydrogen and covalent bonds into a super-helix (Wang *et al.* 2002) undergoes various temperature-dependent and time-dependent changes (Suwa *et al.* 2016). Collagen structure is altered to different degrees at different temperatures (20-200°C), levels of hydration and physical confinement (Miles & Ghelashvili 1999, Vangsness *et al.* 1997, Wang *et al.* 2002, Armstrong *et al*. 2008, Hayashi *et al.* 2012, Gevorkian *et al*. 2013, Suwa *et al*. 2016). At temperatures above 60°C, vibrations between molecules have sufficient energy to break the hydrogen bonds and covalent cross-links. Consequently, the tertiary tri-helical collagen structure is denatured into a random coil (Wang *et al*. 2002) reducing its tensile strength and other physical properties (Stryer 1981, Wang *et al.* 2002). As a result, NaOCl reduces the flexural strength, elastic modulus and visco-elasticity of dentine bars (Sim *et al.* 2001, Grigoratos *et al*. 2001, Ng *et al.* 2020).

Exposure of dentine bars to direct heat and NaOCl, independently and accumulatively, produced moderate changes in quasi-static mechanical properties but more obvious and marked changes in viscoelastic properties measured by dynamic mechanical analysis (DMA) (Karunanayake *et al.* 2019). Immersion of dentine bars in heated NaOCl (60°C or 80°C) significantly increased their visco-elastic behaviour when tested by DMA (Kafantari *et al.* 2019).

It was assumed that the altered mechanical properties of dentine bars would be reflected in altered strain behaviour of whole teeth subjected to non-destructive cyclic loading. This indeed proved to be so, particularly when 5% NaOCl and 17% EDTA were used in alternation (Sim *et al*. 2001, Rajasingham *et al*. 2010) with 30-minute intervals but not 10-minute intervals (Sobhani *et al*. 2010). EDTA by itself did not induce as much damage. Although NaOCl exerted the main damaging effect, the strain behaviour of cyclically-loaded whole teeth was not consistent when irrigated with different NaOCl concentrations (3%, 5.1%, 7.3%), nor the findings entirely explainable (Goldsmith *et al.* 2001). The lack of statistical convergence was assumed to be related to anatomical variations of teeth. It was hypothesised that the extent of "whole tooth" weakening may be dictated by the nature and depth of chemical changes in dentine during and following root canal irrigation, relative to the remaining bulk of unaffected dentine (Ramirez-Bommer *et al*. 2017, Browne *et al.* 2020); the ratio being dependent upon tooth anatomy (Rajasingham *et al.* 2010). However, it remains unclear how the variation in stress-strain characteristics of whole teeth can be fully explained given the diverse and sometimes contradictory findings *in vitro* and the very good clinical survival rates of root-treated teeth over 4 years (Ng *et al.* 2011). Clearly a balance needs to be struck between irrigation protocols that enable effective microbial control without compromising tooth survival (Gulabivala *et al*. 2019).

Given the adopted and established practice of using NaOCl in clinical practice, which may be heated, investigation of its effect on surface strain of whole teeth (albeit measured cervically) under cyclic loading was warranted. The aim of this study was to investigate the effect of irrigation with 5% NaOCl solutions at 21°C, 60°C and 80°C and cyclic loading on (pre-load and loading) tooth surface strain, using saline as control.

Methodology

Collection and storage of teeth

The teeth (n=110) were collected from general dental practices through informed consent (ethical approval reference number: 1301) and stored in 50 mL of 4% formal-saline (Lam & Gulabivala 1996, Jameson *et al.* 1994).

Experimental set-up

A pictogram of the experimental set-up is depicted in figure 1.

Preparation of teeth

Intact, non-carious, mature premolar teeth with single, fused or double roots (n=101) were cleaned of gross debris from their external surfaces. Of these, 36 single-rooted teeth were randomly allocated to groups A1-6 (plus 9 spare), and 56 with mixed anatomy to groups B1-4 (Table 1). Teeth in group B were anatomically characterised using a number of measurements and divided into: single-rooted (n=20), fused-rooted (n=20) and double-rooted (n=16) types and each group further stratified into anatomical subgroups by the measured parameters. Root length, coronal mesio-distal and bucco-lingual width, as well as the thickest dentine from the rim of the access cavity to the periphery of the crown at buccal, lingual, mesial and distal aspects were recorded with a digital caliper (Mitutoyo, UK, Ltd). Digital radiographic images of the teeth in bucco-lingual and mesio-distal dimensions facilitated measurements in these dimensions (CCX Digital Trophy Radiographic Ltd, London, UK), as well as canal width at mid-root, level of canal division (fused-rooted teeth) and level of root-division (double-rooted teeth). After testing for above parameters (using One-way ANOVA, analysis of variance), the teeth were randomly assigned to one of four experimental groups (n=14 per groups B1-4; Table 1) following stratified randomization (n=5 or 4 per root type).

A line drawn around the circumference of the crown (6 mm coronal to the cement-enamel junction [CEJ] for group A, and 4mm coronal to the CEJ for group B), assisted decoronation with a diamond bur in an air-rotor handpiece (KaVo Dental Ltd, Amersham, UK) with copious water-spray. The coronal surface was then flattened perpendicular to the long axis, using a grinder (Struers Knuth Rotor-3 with

Struers waterproof silicon carbide paper, Struers A/S, Copenhagen, Denmark) and water spray. Any remaining enamel was removed without sacrificing dentine.

Canals were accessed and pulps extirpated from all teeth and in group A (plus the 9 spare teeth) prepared to a standard 0.06 taper using Profile® nickel-titanium endodontic instruments (Maillefer Instruments, Ballaigues, Switzerland). Teeth in group B were prepared using ProTaper® F1, F2 and F3 instruments (Dentsply, Maillefer, Ballaigues, Switzerland). Saline irrigation was used for canal preparation in all teeth (groups A & B). Working length was taken from the apical foramen to the coronal reference with a file and canals enlarged apically to ISO size 30 for all teeth, whilst maintained in a hydrated state with saline-soaked tissue paper. The canals were filled with saline to prevent dehydration during subsequent preparation procedures. Following tooth preparation, the apices were sealed with two coats of nail varnish (Rimmel London, London, UK).

Clear acrylic resin (Specifix-20, Struers Epoxy resins, Struers A/S, Copenhagen, Denmark) was poured into plastic circular moulds (2.5 cm height) containing individual teeth, centrally located and axially parallel to the mould, leaving 2mm of exposed root between the CEJ and poured resin (Figure 1). After curing for 24-hr at room temperature and humidity in a fume cupboard, the mould was dismantled, and the base trimmed perpendicular to the long axis and parallel to the flattened occlusal surface of the tooth. The mounted teeth were again maintained in a hydrated state by wrapping in saline-soaked gauze prior to strain gauge bonding (Sobhani *et al.* 2010).

Strain gauge bonding

Constantan strain gauges (Vishay Micro-Measurements Group UK Ltd, Basingstoke, UK) with short copper leads were used: in group A, the resistance was 120 Ω and gauge factor 2.035 (type EA-06-062AP-120, option LE); in group B, the resistance was 350 Ω and gauge factor 2.125 (C2A-06-062LW-350, option LE, Vishay Micro-Measurements Group UK). The gauge-backing was trimmed to enable positioning and appropriate alignment on the tooth. The bonding site was degreased (Vishay Micro-Measurements Group UK Ltd), M-Prep Conditioner A and M-Prep Neutralizer 5A (Vishay Micro-Measurements) applied and the gauge bonded using Micro-Measurements PCT-2M gauge installation tape and M-Bond 200 catalyst and M-Bond 200 adhesive (Vishay Micro-

Measurements) (Figure 1). The strain gauge and wire leads were protected with M-Coat A and M-Coat C (Vishay Micro-Measurements) and cured for 24 hrs at room temperature.

During experimentation, tooth dehydration through evaporation was prevented in group B by filling the canals with saline 2mm below the CEJ with an inverted 23-gauge needle touching the water level to maintain it from a reservoir; the canal orifice was covered with transparent silicone (Memosil, Heraeus Kulzer, Mitsui Chemicals Group, South Bend, IN, USA) holding the needle in place.

Strain indicators and recorder configuration

The Model P3 digital strain indicator was used for group A, and Model P-3500 (Vishay Micro-Measurements, Raleigh, North Carolina, USA) for group B. Having ensured absence of component burn-out by measuring gauge resistance, a 50 mm 330-DFV ribbon cable (Vishay Micro-Measurements Group UK Ltd) was soldered to the strain gauge leads to form a 1 off 3 wire quarter-bridge, using equal lengths of wire for each set-up. The three-wire leads were connected to set-up quarter bridge circuits before activation. The gauge factor was programmed, calibration shunt turned off and channel balanced before strain readings checked to confirm functional integrity. Teeth expressing unstable microstrain readings with a continuous drift were rejected.

Irrigation regimens for experimental groups

Physiological saline (Baxter Healthcare Ltd, Norfolk, UK) and sodium hypochlorite (5% NaOCl) (BDH Laboratory Supplies, Poole, Dorset, UK) were used for irrigation. NaOCl was freshly diluted from commercially available 12% NaOCl stock, verified by iodometric titration. The control and test solutions (60 mL) were stored separately in 120 mL glass containers (Labware Co. Ltd, Hong Kong SAR, China) and heated in a closed system to 60°C using a water bath (Grant Instruments Cambridge Ltd, Barrington, Cambridge, England) when required; the temperature was verified with a thermal sensor (Fluke 179 true RMS multimeter; Fluke UK Ltd, Norfolk, UK).

For group B, the solutions (saline and NaOCl) were heated to the designated temperature of 80°C on a hot-plate (Jenway 1000, Chelmsford, Essex, UK) covered with a plastic lid, constantly agitated and monitored by a thermometer (Fisherbrand, Thermo Fisher Scientific Inc., UK) throughout the experiment.

Load testing of resin-mounted teeth

The tooth-containing acrylic block was secured in a steel receptacle with restraining screws, clamped to the Dartec loading machine crosshead (Zwick Roell, Leominster, Herefordshire, UK) with 1 kN load cell in the upper member. A ball bearing (5 mm diameter) was fixed to the end of the loading arm located over the centre of the access preparation; shim-stock foil (Prestige Dental, Bradford, West Yorkshire, UK) was used to ensure a close and even fit between the loading arm and tooth. Articulating paper (Dentoid Ltd, Huddersfield, UK) was used when the load cycle was initiated to record load distribution photographically (Figure 1). The mounted tooth was not moved laterally during the entire test procedure, except to irrigate, when the crosshead was lowered to create sufficient room.

All teeth received standardised irrigation regimens (Table 1); those in groups A1-2 were irrigated with one test solution 9 times prior to a total of nine loading cycles; those in groups A-3-6 received 18 irrigation regimens with two test solutions applied sequentially, 9 with the first solution and 9 with the second solution; those in group B received 16 irrigation regimens with a single solution.

The coronal portion of each tooth was rubber dam isolated to protect the strain gauge. An initial bolus (0.60 mL) of irrigant was delivered using a Monoject syringe with a 27-gauge needle (Sherwood Medical, St Louis, Missouri, USA) to within 2 mm of the working length, over 20 sec. The irrigant was agitated in the canal with a size 25 Flexo-file® (Maillefer Instruments, Ballaigues, Switzerland) for 100 sec for group A and a sonic device for group B (fabricated from a modified sonic tooth brush [Oral-B^{TM}, Pro-Expert Pulsar^{TM}, Procter & Gamble UK, Weybridge, Surrey, UK] with a tip made from a 27 gauge needle [Sherwood Medical, St Louis, Missouri, USA]). This was repeated 5 times to deliver a total of 3.0 mL of irrigant over 10 min for groups A1-6. After removing gross amounts of the irrigant (suction and paper points) and rubber dam, the tooth was subjected to the loading cycle for exactly 2 min post-irrigation. There were 8 subsequent irrigation cycles for groups A1-2 (total 9), whereas groups A3-6 received 17 subsequent irrigation cycles (total 18) that were consistently initiated at 3 minutes post-loading and 5 minutes post-irrigation. This resulted in a total experimental time of 2.25 hrs for groups A1-2 and 4.5 hrs for groups A3-6, in which saline was used for the first nine irrigation periods followed by the NaOCl solution for the remaining nine irrigation periods.

In group B, the canal was first soaked with saline for 10 minutes and the tooth subjected to the loading cycle. At the end of this, the tooth was isolated with rubber dam and irrigated with a bolus of 0.60 mL delivered over 20 seconds; the solution was agitated with a modified sonic device (Oral-B^{TM}, Pro-Expert Pulsar^{TM}. Procter & Gamble UK, Weybridge, Surrey, UK) for 100 seconds (2 minutes irrigant dwell-time) and the process repeated 15 times to deliver 9.0 mL of irrigant over 30 minutes. The tooth was then subjected to cyclic loading. The entire cycle of irrigation and loading was repeated a further two times giving an overall experimental period of 90 mins.

Each loading period consisted of 3 stages: a) loading from 0-20 N (pre-load); b) 5 cycles of loading from 20-110N and unloading down to 20N at a crosshead speed of 0.1mm/s; and c) unloading from 20-0N. The pre-load was only set once the specimen had been mounted into the steel receptacle and the loading pin aligned. The remainder of the loading regimen was controlled by the Dartec software (Zwick Roell, Leominster, UK). For group B, a pre-load of 5N was applied manually first and then scaled to 20N; furthermore, the un-loading to 20N occurred at a rate of 0.2 Hz.

An initial load cycle was performed prior to irrigation for a baseline value; subsequent load cycles were carried out at fixed time points of three mins post-loading. In Group A, one load cycle (pre-loading, loading and unloading) was completed within 90 sec, whilst in Group B, it was completed in 60 sec, allowing time to re-isolate the tooth with rubber dam and prepare for the next irrigation cycle. The entire experimental period was completed in one sitting for each tooth.

On completion of the experiment, articulating paper (Dentoid Ltd, Huddersfield, UK) was used again to mark the loading areas and photographically recorded for comparison with initial loading zones.

Data recording

The strain indicator recorded strain values every second and the data were documented manually in spreadsheets. Monitoring of microstrain readings from teeth in groups A1-2 during the *non-loading* *period* revealed that they continuously changed synchronously with irrigant delivery and its agitation. Therefore, in groups A3-6, the strain variations during the non-loading periods *were also captured*. After set-up and confirmation of stable microstrain readings, the selected channel was balanced. The peak tooth surface strain was recorded for each of the five loads per cycle. On completion of loading, the minimum peak strain value setting was de-selected to allow non-loading data to be recorded.

The *strain-shift during the non-loading phase* was recorded in groups A3-6 to include the pre-irrigation strain, post-irrigation strain and the pre-load strain. The pre-irrigation strain was recorded following placement of the rubber dam and immediately prior to irrigation. The post-irrigation strain was recorded ten minutes later, immediately following the final agitation of the solution. The pre-load strain was recorded 2 mins later before the load cycle was initiated.

Data analysis

The data were analysed using the STATA® statistics/data analysis software version 15 (StataCorp, College Station, TX, USA). Linear Mixed Models were used to analyse the change in loading tooth surface strain (loading TSS) (µϵ) over the period of irrigation with NaOCl (5%) or saline heated to 60°C or 80°C, adjusted for the pre-irrigation with saline-21°C or saline-60°C for group A, and root type and width of the root at the cemento-enamel junction for group B.

Results

TSS (με) data for Group A

The mean loading TSS (με) for samples in groups A1–6 are presented in table 2: for IP 0, IP 1, IP 9, in groups A1-2; and for IP 0, IP 1, IP 9, IP 10, and IP 18 in groups A3-6. There was wide variation in the baseline loading TSS values. The data for loading tooth surface strain (TSS) against duration, for individual samples in groups A3–6 are depicted in scatter plots in Figures 2-5, including *strain-shift* data.

The non-loading *strain-shift* data revealed a sudden and substantial shift, generally in the positive direction from 0 με, following introduction of saline 21°C (A3, A5) or 60°C (A4, A6) at IP 1 in some samples (A3 teeth 24, 34, 35, 42; A4 teeth 1, 4, 18, 38; A5 teeth 25, 32, 41; A6 all teeth). After this initial change, there was comparatively little further strain change during irrigation with saline in all groups until NaOCl was first introduced. NaOCl delivery incurred a substantial shift in strain during *each* episode of irrigation with NaOCl for the remainder of the experiment in some samples in groups A4 (1, 4, 18, 37) (Figure 3); and A5 (15, 21, 32, 33) (Figure 4). Such strain-shift was also observed in three samples (12, 31, 43) in group A6, albeit of a smaller magnitude (Figure 5). The nature of the shifting strain without obvious mechanical loading led to the adoption of the phrase "strain-shift" to describe the phenomenon. The loading strain was generally in the negative direction.

Linear mixed models (Table 3) revealed irrigation with saline at 21°C (Groups A3&5 pooled data, model 1) or at 60°C (Groups A4&6 pooled data, model 2) from IP0 to IP9 did not result in significant ($p>0.05$) changes in loading TSS. Irrigation with NaOCl at 21°C resulted in significant ($p=0.01$) *increase* in loading TSS over the experimental period (Group A1: IP0 – 1P9; Groups A3&4: IP10 – IP18 pooled data, model 3). In contrast, irrigation with NaOCl at 60°C resulted in a significant ($p=0.001$) *decrease* in loading TSS over time (Group A2: IP0 – 1P9; Groups A5&6: IP10 – IP18 pooled data, model 4). Additionally, the extent of pre-load strain-shift was found to have a negative association with the corresponding loading TSS of teeth after irrigation. However, such a relationship was only significant ($p=0.001$) amongst teeth exposed to saline at 21°C (Table 4).

TSS (µє) data for Group B

Of the 56 teeth, two were excluded (S19 in group B1 [saline at 21°C] and F19 in group B2 [saline 80°C]) (Table 1) because of fracture at baseline loading due to programme malfunction and uncontrolled load application. There were no such malfunctions for other samples. One-way ANOVA revealed no significant ($p>0.01$) difference in any of the anatomical parameters (root length, mesial-width, distal-width, buccal-width, lingual-width) amongst the experimental groups.

Scatter plots of TSS for individual samples over time by each group (B1–4) are presented in figures 6–9. The mean loading TSS for samples in groups B1–4 are presented in table 2 for IP 0, IP 1, IP 2, and IP 3.

Single variable Linear mixed models revealed that “root types” ($p=0.01$) had a significant association with the baseline TSS. The baseline TSS of double-rooted teeth was significantly ($p=0.001$) lower than that of single-rooted teeth. There was no obvious difference between fused- and single-rooted ($p=0.2$) teeth (data not shown). Mesial-thickness ($p=0.001$), and distal-thickness ($p=0.001$) of teeth were also found to have significant association with baseline TSS. The other anatomical parameters: tooth length ($p=0.2$); buccal-thickness ($p=0.5$); and lingual-thickness ($p=0.2$) did not significantly influence TSS.

Linear mixed models (Table 5, models 1&2) incorporating the TSS as a dependent variable, and duration of exposure, root-type and mesial-thickness of teeth as independent variables revealed exposure to saline at 80°C resulted in a significant *increase* in TSS *over time* (Coefficient = 4.58; 95% CI: 1.39, 7.76; $p=0.005$) (Model 2). Such an effect could not be detected in teeth exposed to saline at 21°C (Table 5, model 1). There was, however, no significant change in TSS over the duration of exposure to NaOCl at 21°C ($p=0.7$), or at 80°C ($p=0.2$) (Table 5, models 3&4).

Discussion

This study made the serendipitous discovery from the first two groups (A1, A2) that teeth exhibited characteristic tooth-specific surface strain that appeared to have a maximum limit. This strain could be taken up simply by introducing saline into the root canal system. If that limit was entirely taken-up by saline introduction, the ceiling did not seem to be exceeded, even upon loading. Even more interestingly, introduction of more saline appeared to have no additional effect, that is, pre-load strain caused by saline introduction had a negative effect on the loading strain. Introduction of NaOCl into the canal also caused an immediate change in TSS, however, in the case of this irrigant, TSS was observed upon introduction of *each new bolus*, in contrast to saline. The pre-load TSS again negatively influenced loading-TSS but not to the same extent as for saline, that is, the limiting effect of pre-load strain was less, possibly implying some permanent alteration in dentine structure. These observations have not been reported before and hence a critical assessment of the methodology was performed and is presented to exclude the possibility of artefact.

Studies on mechanical properties of teeth inevitably suffer from the effect of their natural variation and anatomic diversity. Non-stratified tooth allocation, without accounting for anatomy, may lead to confounding and lack of demonstrable (significant) differences between test groups, despite obvious effects being evident at tooth level (Goldsmith *et al*. 2001). The latter study had used a combination of single and double-rooted teeth with mature and immature apices without stratified allocation, whereas other studies (Sim *et al.* 2001, Rajasingham *et al.* 2010, Sobhani *et al.* 2010) only used single-rooted teeth. Sobhani *et al.* (2010) found dentine wall thickness and root length influenced tooth surface strain but the effects of root-pattern have not been reported. Tooth anatomy is difficult to quantify or characterise, particularly from a mechanical perspective but some degree of group homogeneity was achieved in the present study by restricting sample teeth to mature single-rooted premolars in group A and by stratification in group B. The present study found that both mesial and distal dentine thickness were significantly correlated (p=0.001) to TSS at baseline. The mesial wall was significantly (p=0.001) thicker in double-rooted teeth compared to single- or fused-rooted teeth, which may explain why the strain in teeth with double roots was significantly (p=0.001) lower at baseline than the other root types. The effect of remaining dentine thickness at occlusal level on baseline TSS after canal preparation, may be clinically relevant. Care should be taken to keep the access cavity as minimal as possible, as remaining mesial- and distal-thickness has an effect on TSS. Root canal preparation dimensions were standardised within groups, by apical size and taper, to allow the irrigation needle to penetrate consistently to the canal terminus. The method of preparation should be irrelevant as long as dentine stresses are not induced differentially amongst teeth in the process (Adorno *et al.* 2009, Bier *et al.* 2009). A fixed volume of saline was used as an irrigant between files to facilitate a standardised preparation.

The strain measurement model was adopted from previous work (Sim *et al.* 2001, Rajasingham *et al.* 2010, Sobhani *et al.* 2010) but adapted for more precise and continuous tracking of strain changes before and after loading. The present study detected strain change upon rubber dam placement; so damage or direct effect on the strain gauge set-up was avoided by leaving a 2 mm space in the vertical axis.

The single-element strain gauge adopted was of the same type and make used in previous studies (Goldsmith *et al.* 2001, Sobhani *et al.* 2010, Rajasingham *et al.* 2010). Sim *et al.* (2001) had measured both compressive and tensile strain but found the main change to be in the compressive element. The cervical site was chosen because Stress Pattern Analysis by Thermoelastic Emission (SPATE) found it to be a focus of stress concentration during axial loading (Meredith 1992). The “whole tooth” TSS in this study is actually a representation of cervical strain in the vertical plane, since the root is rigidly embedded in resin, allowing mainly the crown to flex. This may also explain why the root length parameter did not reach significance in this study. In the clinical scenario, regionally varying periodontal ligament allows even distribution of loads within it and the alveolar complex (Nikolaus *et al*. 2017), which should be considered for clinical application of the study data.

The present study used the P3 and P3500 Strain Indicator and Recorders (Vishay Measurements Group), which have highly stable measurement circuits, regulated bridge excitation supply, internal dummy and precisely settable gauge factor, enabling measurements of ±0.1% accuracy and 1 microstrain resolution. It enabled strain monitoring throughout the course of irrigation in addition to peak strain measurements on loading. The possibility of flaws in the measurement system to account for strain-drift was carefully and systematically evaluated and excluded.

Although previous studies had used a two-wire circuit (Sim *et al.* 2001, Goldsmith *et al.* 2001, Sobhani *et al.* 2010, Rajasingham *et al.* 2010), the three-wire hook-up used in this study is the recommended configuration for quarter-bridge strain gauge circuits for static strain measurement. Strain gauge measurements depend for their success on their proper and acceptable installation. Each specimen was tested and formally certified by Vishay micro-measurements group for compliance with British Society for Strain Measurement (BSSM) standards for group A.

The conditions of test, including the NaOCl concentration were chosen because 5% is known to induce significant changes in loading-TSS (Sim *et al.* 2001, Sobhani *et al.* 2010, Rajasingham *et al.* 2010). The temperatures of 21°C, 60˚C, and 80°C were chosen based on the maximum temperature that baby-bottle-warmers are likely to reach, accounting for any loss of heat (Macedo *et al.* 2017, de Hemptinne *et al.* 2017, Leonardi *et al*. 2019), as well the reported temperatures at which collagen is denatured (Miles & Ghelashvili 1999, Vangsness *et al.* 1997, Wang *et al.* 2002, Armstrong *et al*. 2008, Hayashi *et al.* 2012, Gevorkian *et al*. 2013, Suwa *et al*. 2016). The duration of number of irrigation cycles (9-18) and total irrigation duration (1.5 – 4.5 hrs) falls within the higher end of the clinically used spectrum but this was designed purposely to detect any effect throughout the spectrum. Saline served as a control irrigant.

The range of load applied (20 N to 110 N) falls within the physiological limits of a human tooth (De Boever *et al.* 1978, Zaslansky *et al*. 2016). The loading-TSS values in this study were generally similar or higher than in other studies (Meredith 1992, Goldsmith *et al.* 2001, Sim *et al.* 2001, Rajasingham *et al.* 2010, Sobhani *et al.* 2010). The differences may be attributed to any one or a combination of differences in methodology, such as the use of immature teeth (Goldsmith *et al.* 2001, Sobhani *et al.* 2010), differences in loading pin and its location and alignment with the tooth (Sim *et al.* 2001), and canal preparation (Sim *et al.* 2001, Goldsmith *et al.* 2001). The wide variation in baseline strain values evident in all experimental groups is attributable to individual tooth differences plus the precision of loading-ball placement and its alignment with the long-axis of the tooth. Based on all these observations, the adopted methodology was considered reliable in the present study.

There was no significant (p>0.05) change in loading TSS (µє) over the experimental period when saline at 21°C was introduced in groups A3&5 or B1 teeth, in line with previous findings (Sobhani *et al.* 2010, Rajasingham *et al.* 2010). The present findings that irrigation with NaOCl (5%) at 21°C in groups A1, A3, and A4, using a single root type resulted in significant (p=0.01) *increase* in TSS, was also in agreement with previous studies (Sim *et al.* 2001, Sobhani *et al.* 2010, Rajasingham *et al.* 2010). On the other hand, the lack of significant (p>0.05) changes in TSS observed amongst the mixed anatomy teeth in group B3 exposed to NaOCl (5%) at 21°C, was in agreement with Goldsmith *et al.* (2001). The latter study had also included mixed groups of teeth, albeit without stratified allocation, where the root-type was found to have a significant (p=0.02) influence on the outcome, masking the effects of the irrigant.

Interestingly, irrigation of the teeth with saline heated to 60°C (groups A4&6) did not display any significant (p>0.05) changes in TSS, whilst irrigation with saline at 80°C (group B3) resulted in a significant (p=0.005) *increase* over time. This appeared to concur with Kafantari *et al.* (2018) who found that immersion of dentine bars in saline at 80˚C significantly increased their visco-elastic behaviour over time. These coupled but independent findings at dentine bar and whole tooth levels suggest that TSS may have been affected *via* denaturation of the tertiary tri-helical structure of collagen beyond 70°C (Wang *et al.* 2002).

Surprisingly, there was significant (p=0.001) *reduction* of TSS over the duration of irrigation with 5% NaOCl at 60°C (Groups A2, A5, A6) but no significant (p>0.05) change in TSS amongst the teeth in the NaOCl 80°C group (B4). Interestingly, the groups with 60° irrigants (A4-6) displayed higher levels of pre-load strain-shift than the group with 21°C irrigants (A3). These seemingly irreconcilable findings may also have an explanation in Kafantari *et al.* (2018), who found a significant reduction in elastic behaviour or an increase in plastic behaviour of dentine bars after exposing them to 5% NaOCl at 60°C or 80°C. In the dentine bar model, the effect of disruption of the interaction between collagen and hydroxyapatite crystals was to cause plastic deformation but in a whole tooth model, the effect maybe to relax the “pre-stress”, such that occlusal loading does not lead to strain changes cervically because of broken “chain links” within collagen and between collagen and hydroxyapatite crystals.

The capacity to de-collagenise dentine is dependent on the volume, concentration and temperature of sodium hypochlorite and its depth of dentinal penetration (Browne *et al.* 2020), which may be affected by root and dentine structure. The lack of significant findings in group B4 could be attributed to more profound changes in collagen (and therefore dentine) properties immediately at the outset, reducing the potential for further change over time.

The loading rate and intervals may also play a role in strain development and relaxation. The present study loaded teeth at a maximum of every 15 mins using an irrigation regimen similar to Sobhani *et al.* (2010), whereas previous studies (Sim *et al.* 2001, Goldsmith *et al.* 2001, Rajasingham *et al.* 2010) loaded teeth following a 30 min irrigation period, as in group B. A longer interval between loading may have provided a safer interval for dentine to recover from accumulative loading stress but there did not appear to be a problem related to it as evidenced by figures 2-5 (Sim *et al.* 2001, Ng *et al.* 2020).

The most interesting outcomes in the present study are depicted in the raw data shown in figures 2-5; they confirm that individual differences between teeth may be the chief confounder in the lack of statistical convergence. Nevertheless, this data revealed that pre-load and loading TSS changes were unique for each tooth, which appeared to exhibit unique “strain limits” that could be reached either by irrigant introduction or tooth loading. Once reached, further change was restricted, unless, presumably, there was permanent structural change induced by thermal, chemical or mechanical means (Luescher *et al.* 1974, Stryer 1981, Osborn 1982, Wang *et al.* 2002, McGee *et al.* 2012, Bertinetti *et al.* 2015).

The present study is to our knowledge, the first to report mechanical changes in dentine measured as “strain-shift” *during the irrigation period*. The term “strain-shift” was coined to describe strain changes without applied loading because the strain appeared to “drift” but it was not a function of the measurement system, nor was it random; it appeared related to definite intra-canal events.

The “strain-shift” data reflected strain changes due to irrigation alone without any loading. The pre-load strain-shift (µɛ) exerted a negative influence on loading TSS during irrigation, regardless of irrigant or temperature. Strain-shift data for saline at 21°C showed a significant increase in strain immediately following irrigant introduction but subsequent irrigation resulted in much smaller changes suggesting some sort of equilibrium or a maximum limit had been reached. Goldsmith *et al.* (2001) also reported this variability in strain recordings but had not measured pre-load strain changes. The strain-shift data for saline at 60°C followed a similar pattern but was of a greater magnitude resulting in smaller strain changes in later irrigation cycles, suggesting a greater proportion of the strain limit had already been "used-up". The observed effect may be explained by some form of chemical change in conformation of collagen-mineral interface that reaches saturation, after which further strain changes may be limited owing to a pre-existing strain-limit having been reached. The effect may be mediated by direct effect of the salt solution on collagen conformation (Luescher *et al.* 1974, McGee *et al*. 2012) or *via* osmotic alteration of water saturation of collagen (Bertinetti *et al.* 2015). The latter showed enormous stress and strain build-up in collagen and associated mineral as a function of dehydration mediated by osmotic pressure. Alternatively, the osmotic effect could also have acted on the unbound water in the dentinal tubules, possibly drawing it out (Kishen & Asundi 2005, Granke *et al*. 2015).

The observations in this study serve to confirm the "pre-stressed laminate" model of tooth structure. The proposed analogy is that of a tooth conceived of as a three-dimensional nano-chain-link structure, which upon having a force applied would distort in the forced direction, taking up any slack in the tooth "structure system". However, further distortion would be limited unless and until the chain-links were plastically distorted or broken. The chain links may be represented in the tooth structure by the interconnection between collagen and hydroxyapatite, as mediated by the presence or absence of bound water. Saline appeared to allow tension and strain to develop (Bertinetti *et al.* 2015), whilst NaOCl appeared to release the tension (presumably through breaking of links), relaxing the structure and preventing transmission of strain from the occlusal loading site to the cervical measurement site.

A change in temperature from either saline at 21°C to NaOCl at 60°C or from saline at 60°C to NaOCl at 21°C demonstrated substantial strain-shifts during each episode of irrigation with NaOCl (at either temperature) for the remainder of the experiment. The strain-shift after irrigation (and before loading) was far greater than any random pre-irrigation strain-shift. These observations are also explainable using the proposed model, through alterations in collagen (Miles & Ghelashvili 1999, Vangsness *et* *al.* 1997, Wang *et al.* 2002, Armstrong *et al*. 2008, Hayashi *et al.* 2012, Gevorkian *et al*. 2013, Suwa *et al*. 2016).

The effects of thermal expansion and contraction of dentine (Lin *et al.* 2010) and the influence of temperature on the strain gauge should be considered (Saw & Messer 1995, Shrestha *et al.* 2010). A temperature rise of 40°C (21°C to 60°C) would have resulted in an error of 0.4%, based on the gauge factor; since the microstrain output was of the order of several hundred, a 0.4% error, would not have had a large impact on the results.

The *rate* of strain-shift, although not measured, was observed to be faster with NaOCl irrigation compared to saline. It was observed that the rate of change with NaOCl at 60°C was far greater than at 21°C, although this was not evident when saline 60°C was used.

The findings of this study offer further and new insight about the behaviour of teeth under specific conditions of irrigant delivery at various temperatures and emphasises the importance of synthesis of data from dentine samples and "whole" tooth models. Whilst the findings do not directly infer increased susceptibility to fracture, they offer new insight about the biomechanics of teeth that may be affected by cyclic loading at low levels.

Conclusions

Tooth anatomy significantly affected its strain characteristics, exhibiting limits within which strain changes occurred. Intra-canal introduction of saline or NaOCl caused non-random strain shifts without loading. Non-random strain shifts during the non-loading phase were related to intra-canal irrigation, including an immediate impact upon first delivery of saline and also most deliveries of NaOCl. Pre-load strain changes negatively influenced loading strain with both irrigants. Irrigation with NaOCl-21°C increased loading tooth strain, as did saline-80°C or NaOCl-80°C but NaOCl-60°C decreased it. A "chain-link" model was proposed to explain the findings and tooth biomechanics.

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Effect of root canal irrigant (sodium hypochlorite & saline) delivery at different temperatures and durations on pre-load and cyclic-loading surface-strain of anatomically different premolars

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Running title: Strain limits of teeth under chemical, thermal and mechanical stresses

Key words: Tooth surface strain, sodium hypochlorite, saline, irrigant temperature, pre-load strain, cyclic loading strain

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Abstract

Aim: To evaluate the effect of NaOCl (5%) and saline (control) irrigant delivery at different temperatures and durations on pre-load and cyclic-loading tooth-surface-strain (TSS) on anatomically different premolars.

Methodology: Single-rooted premolars (n=36), root-canal-prepared in standard manner, were randomly allocated to six irrigation groups: (A1) NaOCl-21°C; (A2) NaOCl-60°C; (A3) saline-21°C then NaOCl-21°C; (A4) saline-60°C then NaOCl-21°C; (A5) saline-21°C then NaOCl-60°C; (A6) saline-60°C then NaOCl-60°C. A1-2 received nine 10-min irrigation periods (IP) with NaOCl; A3-6 received nine 10-minute IP with saline, followed by 9 IP with NaOCl at different temperature combinations. Premolars (n=56) with single, fused or double roots prepared by standard protocol, were stratified and randomly allocated to: (B1) saline-21°C; (B2) saline-80°C; (B3) NaOCl-21°C; (B4) NaOCl-80°C. TSS (μɛ) was recorded pre-irrigation, post-irrigation and pre-load for each IP and during cyclic loading 2 min after each IP, over 30-274 min, using strain-gauges. Generalised linear mixed models were used for analysis.

Results: Baseline TSS in double-rooted premolars was significantly ($p=0.001$) lower than in single/fused-rooted-premolars; and affected by mesial-wall-thickness ($p=0.005$). There was significant increase in loading-TSS (μɛ) after NaOCl-21°C irrigation ($p=0.01$) but decrease after NaOCl-60°C irrigation ($p=0.001$). TSS also increased significantly ($p=0.005$) after Saline-80°C irrigation. *Pre-load* “strain-shift” was noted only upon first saline delivery but every-time with NaOCl. Strain-shift negatively influenced loading-TSS after saline or NaOCl irrigation (A3-6) but was only significant for saline-21°C.

Conclusions: Tooth anatomy significantly affected its strain characteristics, exhibiting limits within which strain changes occurred. Intra-canal introduction of saline or NaOCl caused non-random strain shifts without loading. Irrigation with NaOCl-21°C increased loading tooth strain, as did saline-80°C or NaOCl-80°C but NaOCl-60°C decreased it. A “chain-link” model was proposed to explain the findings and tooth biomechanics.

INTRODUCTION

Teeth appear to be simple structures on a gross scale but display astounding beauty and complexity at micro and nanoscopic scales that reflects their functional and mechanical behaviour (Currey 1999). The “pre-stressed laminate” behaviour of intact teeth was hypothesised long before proof of mechanisms emerged (Tidmarsh 1976). Such “pre-stress” maybe due to the dynamic interaction between collagen molecules, nanometre-sized mineral particles and the water content in dentine (Bertinetti *et al.* 2015, Forien *et al.* 2016). These interactions provide strengthening and toughening mechanisms designed to prevent crack propagation in a non-repairing hydrated mineral tissue (Zaslansky *et al.* 2016). The response to tooth-loading is also moderated by tooth architecture and its periodontal support structure (Naveh *et al.* 2012, Nikolaus *et al.* 2017). Despite the evolving insight about tooth biomechanics, much still remains to be discovered about their fatigue strength and long-term behaviour.

Disease and restorative procedures weaken teeth by disrupting their architecture and ability to respond favourably to loading (Vukicevic *et al.* 2015). Root canal treatment is a procedure used to control root canal infection and periapical disease by irrigation of its fine capillary-like canals using sodium hypochlorite (Gulabivala *et al.* 2019). Root canal treatment may incur further tooth structure disruption ~~loss~~ through canal preparation (Lang *et al.* 2006) and chemical (Sim *et al.* 2001, Grigoratos *et al.* 2001, Rajasingham *et al.* 2010) or thermal (Karunanayake *et al.* 2018, Kafantari *et al.* 2018) damage by alteration of~~ing~~ compositional (O’Driscoll *et al.* 2002, Pascon *et al.* 2012, Ramirez-Bommer *et al.* 2017) and mechanical properties (Sim *et al.* 2001, Grigoratos *et al.* 2001, Rajasingham *et al.* 2010).

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The commonly adopted sodium hypochlorite (NaOCl) irrigant for root canal treatment causes chemical damage to dentine (O’Driscoll *et al.* 2002). Since both antimicrobial (Cunningham & Joseph 1980, Sirtes *et al.* 2005) and tissue-dissolving (Cunningham & Balekjian 1980, Abou-Rass & Oglesby 1981) properties are enhanced by higher temperature, *heated NaOCl* has been advocated for enhancing root canal debridement potential during clinical use (Ruddle 1994, Castelluci 2004). Heating devices for endodontic irrigating syringes (EndoWarmer® [Albrina, Forli, Italy], Syringe-

Warming Device [Keydent, Vaterstetten, Germany]) allow NaOCl to be heated to as high as 80°C before introduction into the root canal system or alternatively other strategies involve intra-canal heating of solutions (de Hemptinne *et al.* 2017, Leonardi *et al*. 2019). Although, the effect of heat and *heated* NaOCl on the mechanical properties of dentine bars has been determined (Karunanayake *et al.* 2018, Kafantari *et al.* 2018), its effect on whole teeth (Sim *et al.* 2001) during root canal irrigation has not ~~(Sim *et al.* 2001)~~.

Heat may induce evaporation of unbound dentine water as well as loss of bound water at temperatures above 200°C (Helfer *et al*. 1972). Since water is integral to the viscoelastic properties of dentine (Craig & Peyton 1958), its loss would compromise the mechanical properties of teeth (Huang *et al.* 1992, Jameson *et al.* 1993, Kishen *et al.* 2001).

Heat may also compromise dentine properties directly by altering intra-dentinal collagen properties. Collagen consisting of 3 helical polypeptide chains bound together by hydrogen and covalent bonds into a super-helix (Wang *et al.* 2002) undergoes various temperature-dependent and time-dependent changes (Suwa *et al*. 2016). Collagen structure is altered to different degrees at different temperatures (20-200°C), levels of hydration and physical confinement (Miles & Ghelashvili 1999, Vangsness *et al.* 1997, Wang *et al.* 2002, Armstrong *et al*. 2008, Hayashi *et al.* 2012, Gevorkian *et al*. 2013, Suwa *et al*. 2016). At temperatures above 60°C, vibrations between molecules have sufficient energy to break the hydrogen bonds and covalent cross-links. Consequently, the tertiary tri-helical collagen structure is denatured into a random coil (Wang *et al*. 2002) reducing its tensile strength and other physical properties (Stryer 1981, Wang *et al.* 2002). As a result, NaOCl reduces the flexural strength, elastic modulus and visco-elasticity of dentine bars (Sim *et al*. 2001, Grigoratos *et al*. 2001, Ng *et al.* 2020).

Exposure of dentine bars to direct heat and NaOCl, independently and accumulatively, produced moderate changes in quasi-static mechanical properties but more obvious and marked changes in viscoelastic properties measured by dynamic mechanical analysis (DMA) (Karunanayake *et al.* 2019). Immersion of dentine bars in heated NaOCl (60°C or 80°C) significantly increased their visco-elastic behaviour when tested by DMA (Kafantari *et al.* 2019).

It was assumed that the altered mechanical properties of dentine bars would be reflected in altered strain behaviour of whole teeth subjected to non-destructive cyclic loading. This indeed proved to be so, particularly when 5% NaOCl and 17% EDTA were used in alternation (Sim *et al*. 2001, Rajasingham *et al*. 2010) with 30-minute intervals but not 10-minute intervals (~~,~~ Sobhani *et al*. 2010). EDTA by itself did not induce as much damage. Although NaOCl exerted the main damaging effect~~However~~, the strain behaviour of cyclically-loaded whole teeth was not consistent when irrigated with different NaOCl concentrations (3%, 5.1%, 7.3%), nor the findings entirely explainable (Goldsmith *et al.* 2001). The lack of statistical convergence was assumed to be related to anatomical variations of teeth. It was hypothesised that the extent of "whole tooth" weakening may be dictated by the nature and depth of chemical changes in dentine during and following root canal irrigation, relative to the remaining bulk of unaffected dentine (Ramirez-Bommer *et al*. 2017, Browne *et al.* 2020); the ratio being dependent upon tooth anatomy (Rajasingham *et al.* 2010). However, it remains unclear how the variation in stress-strain characteristics of whole teeth can be fully explained given the diverse and sometimes contradictory findings *in vitro* and the very good clinical survival rates of root-treated teeth over 4 years (Ng *et al.* 2011). Clearly a balance needs to be struck between irrigation protocols that enable effective microbial control without compromising tooth survival (Gulabivala *et al*. 2019).

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Given the adopted and established practice of using ~~heated~~ NaOCl in clinical practice, which may be heated, investigation of its effect on surface strain of whole teeth (albeit measured cervically) under cyclic loading was warranted. The aim of this study was to investigate the effect of irrigation with 5% NaOCl solutions at 21°C, 60°C and 80°C and cyclic loading on (pre-load and~~)~~ loading) tooth surface strain, using saline as control.

Methodology

Collection and storage of teeth

The teeth (n=110) were collected from general dental practices through informed consent (ethical approval reference number: 1301) and stored in 50 mL of 4% formal-saline (Lam & Gulabivala 1996, Jameson *et al.* 1994).

Experimental set-up

A pictogram of the experimental set-up is depicted in figure 1.

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Preparation of teeth

Intact, non-carious, mature premolar teeth with single, fused or double roots (n=101) were cleaned of gross debris from their external surfaces. Of these, 36 single-rooted teeth were randomly allocated to groups A1-6 (plus 9 spare), and 56 with mixed anatomy to groups B1-4 (Table 1). Teeth in group B were anatomically characterised using a number of measurements and divided into: single-rooted (n=20), fused-rooted (n=20) and double-rooted (n=16) types and each group further stratified into anatomical subgroups by the measured parameters. Root length, coronal mesio-distal and bucco-lingual width, as well as the thickest dentine from the rim of the access cavity to the periphery of the crown at buccal, lingual, mesial and distal aspects were recorded with a digital caliper (Mitutoyo, UK, Ltd). Digital radiographic images of the teeth in bucco-lingual and mesio-distal dimensions facilitated measurements in these dimensions (CCX Digital Trophy Radiographic Ltd, London, UK), as well as canal width at mid-root, level of canal division (fused-rooted teeth) and level of root-division (double-rooted teeth). After testing for above parameters (using One-way ANOVA, analysis of variance), the teeth were randomly assigned to one of four experimental groups (n=14 per groups B1-4; Table 1) following stratified randomization (n=5 or 4 per root type).

A line drawn around the circumference of the crown (6 mm coronal to the cement-enamel junction [CEJ] for group A, and 4mm coronal to the CEJ for group B), assisted decoronation with a diamond bur in an air-rotor handpiece (KaVo Dental Ltd, Amersham, UK) with copious water-spray. The coronal surface was then flattened perpendicular to the long axis, using a grinder (Struers Knuth Rotor-3 with

Struers waterproof silicon carbide paper, Struers A/S, Copenhagen, Denmark) and water spray. Any remaining enamel was removed without sacrificing dentine.

Canals were accessed and pulps extirpated from all teeth and in group A (plus the 9 spare teeth) prepared to a standard 0.06 taper using Profile® nickel-titanium endodontic instruments (Maillefer Instruments, Ballaigues, Switzerland). Teeth in group B were prepared using ProTaper® F1, F2 and F3 instruments (Dentsply, Maillefer, Ballaigues, Switzerland). Saline irrigation was used for canal preparation in all teeth (groups A & B). Working length was taken from the apical foramen to the coronal reference with a file and canals enlarged apically to ISO size 30 for all teeth, whilst maintained in a hydrated state with saline-soaked tissue paper. The canals were filled with saline to prevent dehydration during subsequent preparation procedures. Following tooth preparation, the apices were sealed with two coats of nail varnish (Rimmel London, London, UK).

Clear acrylic resin (Specifix-20, Struers Epoxy resins, Struers A/S, Copenhagen, Denmark) was poured into plastic circular moulds (2.5 cm height) containing individual teeth, centrally located and axially parallel to the mould, leaving 2mm of exposed root between the CEJ and poured resin (Figure 1). After curing for 24-hr at room temperature and humidity in a fume cupboard, the mould was dismantled, and the base trimmed perpendicular to the long axis and parallel to the flattened occlusal surface of the tooth. The mounted teeth were again maintained in a hydrated state by wrapping in saline-soaked gauze prior to strain gauge bonding (Sobhani *et al.* 2010).

Strain gauge bonding

Constantan strain gauges (Vishay Micro-Measurements Group UK Ltd, Basingstoke, UK) with short copper leads were used: in group A, the resistance was 120 Ω and gauge factor 2.035 (type EA-06-062AP-120, option LE); in group B, the resistance was 350 Ω and gauge factor 2.125 (C2A-06-062LW-350, option LE, Vishay Micro-Measurements Group UK). The gauge-backing was trimmed to enable positioning and appropriate alignment on the tooth. The bonding site was degreased (Vishay Micro-Measurements Group UK Ltd), M-Prep Conditioner A and M-Prep Neutralizer 5A (Vishay Micro-Measurements) applied and the gauge bonded using Micro-Measurements PCT-2M gauge installation tape and M-Bond 200 catalyst and M-Bond 200 adhesive (Vishay Micro-

Measurements) (Figure 1). The strain gauge and wire leads were protected with M-Coat A and M-Coat C (Vishay Micro-Measurements) and cured for 24 hrs at room temperature.

During experimentation, tooth dehydration through evaporation was prevented in group B by filling the canals with saline 2mm below the CEJ with an inverted 23-gauge needle touching the water level to maintain it from a reservoir; the canal orifice was covered with transparent silicone (Memosil, Heraeus Kulzer, Mitsui Chemicals Group, South Bend, IN, USA) holding the needle in place.

Strain indicators and recorder configuration

The Model P3 digital strain indicator was used for group A, and Model P-3500 (Vishay Micro-Measurements, Raleigh, North Carolina, USA) for group B. Having ensured absence of component burn-out by measuring gauge resistance, a 50 mm 330-DFV ribbon cable (Vishay Micro-Measurements Group UK Ltd) was soldered to the strain gauge leads to form a 1 off 3 wire quarter-bridge, using equal lengths of wire for each set-up. The three-wire leads were connected to set-up quarter bridge circuits before activation. The gauge factor was programmed, calibration shunt turned off and channel balanced before strain readings checked to confirm functional integrity. Teeth expressing unstable microstrain readings with a continuous drift were rejected.

Irrigation regimens for experimental groups

Physiological saline (Baxter Healthcare Ltd, Norfolk, UK) and sodium hypochlorite (5% NaOCl) (BDH Laboratory Supplies, Poole, Dorset, UK) were used for irrigation. NaOCl was freshly diluted from commercially available 12% NaOCl stock, verified by iodometric titration. The control and test solutions (60 mL) were stored separately in 120 mL glass containers (Labware Co. Ltd, Hong Kong SAR, China) and heated in a closed system to 60°C using a water bath (Grant Instruments Cambridge Ltd, Barrington, Cambridge, England) when required; the temperature was verified with a thermal sensor (Fluke 179 true RMS multimeter; Fluke UK Ltd, Norfolk, UK).

For group B, the solutions (saline and NaOCl) were heated to the designated temperature of 80°C on a hot-plate (Jenway 1000, Chelmsford, Essex, UK) covered with a plastic lid, constantly agitated and monitored by a thermometer (Fisherbrand, Thermo Fisher Scientific Inc., UK) throughout the experiment.

Load testing of resin-mounted teeth

The tooth-containing acrylic block was secured in a steel receptacle with restraining screws, clamped to the Dartec loading machine crosshead (Zwick Roell, Leominster, Herefordshire, UK) with 1 kN load cell in the upper member. A ball bearing (5 mm diameter) was fixed to the end of the loading arm located over the centre of the access preparation; shim-stock foil (Prestige Dental, Bradford, West Yorkshire, UK) was used to ensure a close and even fit between the loading arm and tooth. Articulating paper (Dentoid Ltd, Huddersfield, UK) was used when the load cycle was initiated to record load distribution photographically (Figure 1). The mounted tooth was not moved laterally during the entire test procedure, except to irrigate, when the crosshead was lowered to create sufficient room.

All teeth received standardised irrigation regimens (Table 1); those in groups A1-2 were irrigated with one test solution 9 times prior to a total of nine loading cycles; those in groups A-3-6 received 18 irrigation regimens with two test solutions applied sequentially, 9 with the first solution and 9 with the second solution; those in group B received 16 irrigation regimens with a single solution.

The coronal portion of each tooth was rubber dam isolated to protect the strain gauge. An initial bolus (0.60 mL) of irrigant was delivered using a Monoject syringe with a 27-gauge needle (Sherwood Medical, St Louis, Missouri, USA) to within 2 mm of the working length, over 20 sec. The irrigant was agitated in the canal with a size 25 Flexo-file® (Maillefer Instruments, Ballaigues, Switzerland) for 100 sec for group A and a sonic device for group B (fabricated from a modified sonic tooth brush [Oral-B^{TM}, Pro-Expert Pulsar^{TM}, Procter & Gamble UK, Weybridge, Surrey, UK] with a tip made from a 27 gauge needle [Sherwood Medical, St Louis, Missouri, USA]). This was repeated 5 times to deliver a total of 3.0 mL of irrigant over 10 min for groups A1-6. After removing gross amounts of the irrigant (suction and paper points) and rubber dam, the tooth was subjected to the loading cycle for exactly 2 min post-irrigation. There were 8 subsequent irrigation cycles for groups A1-2 (total 9), whereas groups A3-6 received 17 subsequent irrigation cycles (total 18) that were consistently initiated at 3 minutes post-loading and 5 minutes post-irrigation. This resulted in a total experimental time of 2.25 hrs for groups A1-2 and 4.5 hrs for groups A3-6, in which saline was used for the first nine irrigation periods followed by the NaOCl solution for the remaining nine irrigation periods.

In group B, the canal was first soaked with saline for 10 minutes and the tooth subjected to the loading cycle. At the end of this, the tooth was isolated with rubber dam and irrigated with a bolus of 0.60 mL delivered over 20 seconds; the solution was agitated with a modified sonic device (Oral-B^{TM}, Pro-Expert Pulsar^{TM}. Procter & Gamble UK, Weybridge, Surrey, UK) for 100 seconds (2 minutes irrigant dwell-time) and the process repeated 15 times to deliver 9.0 mL of irrigant over 30 minutes. The tooth was then subjected to cyclic loading. The entire cycle of irrigation and loading was repeated a further two times giving an overall experimental period of 90 mins.

Each loading period consisted of 3 stages: a) loading from 0-20 N (pre-load); b) 5 cycles of loading from 20-110N and unloading down to 20N at a crosshead speed of 0.1mm/s; and c) unloading from 20-0N. The pre-load was only set once the specimen had been mounted into the steel receptacle and the loading pin aligned. The remainder of the loading regimen was controlled by the Dartec software (Zwick Roell, Leominster, UK). For group B, a pre-load of 5N was applied manually first and then scaled to 20N; furthermore, the un-loading to 20N occurred at a rate of 0.2 Hz.

An initial load cycle was performed prior to irrigation for a baseline value; subsequent load cycles were carried out at fixed time points of three mins post-loading. In Group A, one load cycle (pre-loading, loading and unloading) was completed within 90 sec, whilst in Group B, it was completed in 60 sec, allowing time to re-isolate the tooth with rubber dam and prepare for the next irrigation cycle. The entire experimental period was completed in one sitting for each tooth.

On completion of the experiment, articulating paper (Dentoid Ltd, Huddersfield, UK) was used again to mark the loading areas and photographically recorded for comparison with initial loading zones.

Data recording

The strain indicator recorded strain values every second and the data were documented manually in spreadsheets. Monitoring of microstrain readings from teeth in groups A1-2 during the *non-loading* *period* revealed that they continuously changed synchronously with irrigant delivery and its agitation. Therefore, in groups A3-6, the strain variations during the non-loading periods *were also captured*. After set-up and confirmation of stable microstrain readings, the selected channel was balanced. The peak tooth surface strain was recorded for each of the five loads per cycle. On completion of loading, the minimum peak strain value setting was de-selected to allow non-loading data to be recorded.

The *strain-shift during the non-loading phase* was recorded in groups A3-6 to include the pre-irrigation strain, post-irrigation strain and the pre-load strain. The pre-irrigation strain was recorded following placement of the rubber dam and immediately prior to irrigation. The post-irrigation strain was recorded ten minutes later, immediately following the final agitation of the solution. The pre-load strain was recorded 2 mins later before the load cycle was initiated.

Data analysis

The data were analysed using the STATA® statistics/data analysis software version 15 (StataCorp, College Station, TX, USA). Linear Mixed Models were used to analyse the change in loading tooth surface strain (loading TSS) (μϵ) over the period of irrigation with NaOCl (5%) or saline heated to 60°C or 80°C, adjusted for the pre-irrigation with saline-21°C or saline-60°C for group A, and root type and width of the root at the cemento-enamel junction for group B.

Results

TSS (µє) data for Group A

The mean loading TSS (µє) for samples in groups A1–6 are presented in table 2: for IP 0, IP 1, IP 9, in groups A1-2; and for IP 0, IP 1, IP 9, IP 10, and IP 18 in groups A3-6. There was wide variation in the baseline loading TSS values. The data for loading tooth surface strain (TSS) against duration, for individual samples in groups A3–6 are depicted in scatter plots in Figures 2~~1~~-5~~4~~, including *strain-shift* data.

The non-loading *strain-shift* data revealed a sudden and substantial shift, generally in the positive direction from 0 µє, following introduction of saline 21°C (A3, A5) or 60°C (A4, A6) at IP 1 in some samples (A3 teeth 24, 34, 35, 42; A4 teeth 1, 4, 18, 38; A5 teeth 25, 32, 41; A6 all teeth). After this initial change, there was comparatively little further strain change during irrigation with saline in all groups until NaOCl was first introduced. NaOCl delivery incurred a substantial shift in strain during *each* episode of irrigation with NaOCl for the remainder of the experiment in some samples in groups A4 (1, 4, 18, 37) (Figure 3~~2~~); and A5 (15, 21, 32, 33) (Figure 4~~3~~). Such strain-shift was also observed in three samples (12, 31, 43) in group A6, albeit of a smaller magnitude (Figure 5~~4~~). The nature of the shifting strain without obvious mechanical loading led to the adoption of the phrase "strain-shift" to describe the phenomenon. The loading strain was generally in the negative direction.

Linear mixed models (Table 3) revealed irrigation with saline at 21°C (Groups A3&5 pooled data, model 1) or at 60°C (Groups A4&6 pooled data, model 2) from IP0 to IP9 did not result in significant ($p>0.05$) changes in loading TSS. Irrigation with NaOCl at 21°C resulted in significant ($p=0.01$) *increase* in loading TSS over the experimental period (Group A1: IP0 – 1P9; Groups A3&4: IP10 – IP18 pooled data, model 3). In contrast, irrigation with NaOCl at 60°C resulted in a significant ($p=0.001$) *decrease* in loading TSS over time (Group A2: IP0 – 1P9; Groups A5&6: IP10 – IP18 pooled data, model 4). Additionally, the extent of pre-load strain-shift was found to have a negative association with the corresponding loading TSS of teeth after irrigation. However, such a relationship was only significant ($p=0.001$) amongst teeth exposed to saline at 21°C (Table 4).

TSS (µϵ) data for Group B

Of the 56 teeth, two were excluded (S19 in group B1 [saline at 21°C] and F19 in group B2 [saline 80°C]) (Table 1) because of fracture at baseline loading due to programme malfunction and uncontrolled load application. There were no such malfunctions for other samples. One-way ANOVA revealed no significant (p>0.01) difference in any of the anatomical parameters (root length, mesial-width, distal-width, buccal-width, lingual-width) amongst the experimental groups.

Scatter plots of TSS for individual samples over time by each group (B1–4) are presented in figures 6~~5~~–9~~8~~. The mean loading TSS for samples in groups B1–4 are presented in table 2 for IP 0, IP 1, IP 2, and IP 3.

Single variable Linear mixed models revealed that “root types” (p=0.01) had a significant association with the baseline TSS. The baseline TSS of double-rooted teeth was significantly (p=0.001) lower than that of single-rooted teeth. There was no obvious difference between fused- and single-rooted (p=0.2) teeth (data not shown). Mesial-thickness (p=0.001), and distal-thickness (p=0.001) of teeth were also found to have significant association with baseline TSS. The other anatomical parameters: tooth length (p=0.2); buccal-thickness (p=0.5); and lingual-thickness (p=0.2) did not significantly influence TSS.

Linear mixed models (Table 5, models 1&2) incorporating the TSS as a dependent variable, and duration of exposure, root-type and mesial-thickness of teeth as independent variables revealed exposure to saline at 80°C resulted in a significant *increase* in TSS *over time* (Coefficient = 4.58; 95% CI: 1.39, 7.76; p=0.005) (Model 2). Such an effect could not be detected in teeth exposed to saline at 21°C (Table 5, model 1). There was, however, no significant change in TSS over the duration of exposure to NaOCl at 21°C (p=0.7), or at 80°C (p=0.2) (Table 5, models 3&4).

Discussion

This study made the serendipitous discovery from the first two groups (A1, A2) that teeth exhibited characteristic tooth-specific surface strain that appeared to have a maximum limit. This strain could be taken up simply by introducing saline into the root canal system. If that limit was entirely taken-up by saline introduction, the ceiling did not seem to be exceeded, even upon loading. Even more interestingly, introduction of more saline appeared to have no additional effect, that is, pre-load strain caused by saline introduction had a negative effect on the loading strain. Introduction of NaOCl into the canal also caused an immediate change in TSS, however, in the case of this irrigant, TSS was observed upon introduction of *each new bolus*, in contrast to saline. The pre-load TSS again negatively influenced loading-TSS but not to the same extent as for saline, that is, the limiting effect of pre-load strain was less, possibly implying some permanent alteration in dentine structure. These observations have not been reported before and hence a critical assessment of the methodology was performed and is presented to exclude the possibility of artefact.

Studies on mechanical properties of teeth inevitably suffer from the effect of their natural variation and anatomic diversity. Non-stratified tooth allocation, without accounting for anatomy, may lead to confounding and lack of demonstrable (significant) differences between test groups, despite obvious effects being evident at tooth level (Goldsmith *et al*. 2001). The latter study had used a combination of single and double-rooted teeth with mature and immature apices without stratified allocation, whereas other studies (Sim *et al.* 2001, Rajasingham *et al.* 2010, Sobhani *et al.* 2010) only used single-rooted teeth. Sobhani *et al.* (2010) found dentine wall thickness and root length influenced tooth surface strain but the effects of root-pattern have not been reported. Tooth anatomy is difficult to quantify or characterise, particularly from a mechanical perspective but some degree of group homogeneity was achieved in the present study by restricting sample teeth to mature single-rooted premolars in group A and by stratification in group B. The present study found that both mesial and distal dentine thickness were significantly correlated (p=0.001) to TSS at baseline. The mesial wall was significantly (p=0.001) thicker in double-rooted teeth compared to single- or fused-rooted teeth, which may explain why the strain in teeth with double roots was significantly (p=0.001) lower at baseline than the other root types. The effect of remaining dentine thickness at occlusal level on baseline TSS after canal preparation, may be clinically relevant. Care should be taken to keep the access cavity as minimal as possible, as remaining mesial- and distal-thickness has an effect on TSS. Root canal preparation dimensions were standardised within groups, by apical size and taper, to allow the irrigation needle to penetrate consistently to the canal terminus. The method of preparation should be irrelevant as long as dentine stresses are not induced differentially amongst teeth in the process (Adorno *et al.* 2009, Bier *et al.* 2009). A fixed volume of saline was used as an irrigant between files to facilitate a standardised preparation.

The strain measurement model was adopted from previous work (Sim *et al.* 2001, Rajasingham *et al.* 2010, Sobhani *et al.* 2010) but adapted for more precise and continuous tracking of strain changes before and after loading. The present study detected strain change upon rubber dam placement; so damage or direct effect on the strain gauge set-up was avoided by leaving a 2 mm space in the vertical axis.

The single-element strain gauge adopted was of the same type and make used in previous studies (Goldsmith *et al.* 2001, Sobhani *et al.* 2010, Rajasingham *et al.* 2010). Sim *et al.* (2001) had measured both compressive and tensile strain but found the main change to be in the compressive element. The cervical site was chosen because Stress Pattern Analysis by Thermoelastic Emission (SPATE) found it to be a focus of stress concentration during axial loading (Meredith 1992). The “whole tooth” TSS in this study is actually a representation of cervical strain in the vertical plane, since the root is rigidly embedded in resin, allowing mainly the crown to flex. This may also explain why the root length parameter did not reach significance in this study. In the clinical scenario, regionally varying periodontal ligament allows even distribution of loads within it and the alveolar complex (Nikolaus *et al*. 2017), which should be considered for clinical application of the study data.

The present study used the P3 and P3500 Strain Indicator and Recorders (Vishay Measurements Group), which have highly stable measurement circuits, regulated bridge excitation supply, internal dummy and precisely settable gauge factor, enabling measurements of ±0.1% accuracy and 1 microstrain resolution. It enabled strain monitoring throughout the course of irrigation in addition to peak strain measurements on loading. The possibility of flaws in the measurement system to account for strain-drift was carefully and systematically evaluated and excluded.

Although previous studies had used a two-wire circuit (Sim *et al.* 2001, Goldsmith *et al.* 2001, Sobhani *et al.* 2010, Rajasingham *et al.* 2010), the three-wire hook-up used in this study is the recommended configuration for quarter-bridge strain gauge circuits for static strain measurement. Strain gauge measurements depend for their success on their proper and acceptable installation. Each specimen was tested and formally certified by Vishay micro-measurements group for compliance with British Society for Strain Measurement (BSSM) standards for group A.

The conditions of test, including the NaOCl concentration were chosen because 5% is known to induce significant changes in loading-TSS (Sim *et al.* 2001, Sobhani *et al.* 2010, Rajasingham *et al.* 2010). The temperatures of 21°C, 60˚C, and 80°C were chosen based on the maximum temperature that baby-bottle-warmers are likely to reach, accounting for any loss of heat (Macedo *et al.* 2017, de Hemptinne *et al.* 2017, Leonardi *et al*. 2019), as well the reported temperatures at which collagen is denatured (Miles & Ghelashvili 1999, Vangsness *et al.* 1997, Wang *et al.* 2002, Armstrong *et al*. 2008, Hayashi *et al.* 2012, Gevorkian *et al*. 2013, Suwa *et al*. 2016). The duration of number of irrigation cycles (9-18) and total irrigation duration (1.5 – 4.5 hrs) falls within the higher end of the clinically used spectrum but this was designed purposely to detect any effect throughout the spectrum. Saline served as a control irrigant.

The range of load applied (20 N to 110 N) falls within the physiological limits of a human tooth (De Boever *et al.* 1978, Zaslansky *et al*. 2016). The loading-TSS values in this study were generally similar or higher than in other studies (Meredith 1992, Goldsmith *et al.* 2001, Sim *et al.* 2001, Rajasingham *et al.* 2010, Sobhani *et al.* 2010). The differences may be attributed to any one or a combination of differences in methodology, such as the use of immature teeth (Goldsmith *et al.* 2001, Sobhani *et al.* 2010), differences in loading pin and its location and alignment with the tooth (Sim *et al.* 2001), and canal preparation (Sim *et al.* 2001, Goldsmith *et al.* 2001). The wide variation in baseline strain values evident in all experimental groups is attributable to individual tooth differences plus the precision of loading-ball placement and its alignment with the long-axis of the tooth. Based on all these observations, the adopted methodology was considered reliable in the present study.

There was no significant (p>0.05) change in loading TSS (µɛ) over the experimental period when saline at 21°C was introduced in groups A3&5 or B1 teeth, in line with previous findings (Sobhani *et al.* 2010, Rajasingham *et al.* 2010). The present findings that irrigation with NaOCl (5%) at 21°C in groups A1, A3, and A4, using a single root type resulted in significant (p=0.01) *increase* in TSS, was also in agreement with previous studies (Sim *et al.* 2001, Sobhani *et al.* 2010, Rajasingham *et al.* 2010). On the other hand, the lack of significant (p>0.05) changes in TSS observed amongst the mixed anatomy teeth in group B3 exposed to NaOCl (5%) at 21°C, was in agreement with Goldsmith *et al.* (2001). The latter study had also included mixed groups of teeth, albeit without stratified allocation, where the root-type was found to have a significant (p=0.02) influence on the outcome, masking the effects of the irrigant.

Interestingly, irrigation of the teeth with saline heated to 60°C (groups A4&6) did not display any significant (p>0.05) changes in TSS, whilst irrigation with saline at 80°C (group B3) resulted in a significant (p=0.005) *increase* over time. This appeared to concur with Kafantari *et al.* (2018) who found that immersion of dentine bars in saline at 80˚C significantly increased their visco-elastic behaviour over time. These coupled but independent findings at dentine bar and whole tooth levels suggest that TSS may have been affected *via* denaturation of the tertiary tri-helical structure of collagen beyond 70°C (Wang *et al.* 2002).

Surprisingly, there was significant (p=0.001) *reduction* of TSS over the duration of irrigation with 5% NaOCl at 60°C (Groups A2, A5, A6) but no significant (p>0.05) change in TSS amongst the teeth in the NaOCl 80°C group (B4). Interestingly, the groups with 60° irrigants (A4-6) displayed higher levels of pre-load strain-shift than the group with 21°C irrigants (A3). These seemingly irreconcilable findings may also have an explanation in Kafantari *et al.* (2018), who found a significant reduction in elastic behaviour or an increase in plastic behaviour of dentine bars after exposing them to 5% NaOCl at 60°C or 80°C. In the dentine bar model, the effect of disruption of the interaction between collagen and hydroxyapatite crystals was to cause plastic deformation but in a whole tooth model, the effect maybe to relax the “pre-stress”, such that occlusal loading does not lead to strain changes cervically because of broken “chain links” within collagen and between collagen and hydroxyapatite crystals.

The capacity to de-collagenise dentine is dependent on the volume, concentration and temperature of sodium hypochlorite and its depth of dentinal penetration (Browne *et al.* 2020), which may be affected by root and dentine structure. The lack of significant findings in group B4 could be attributed to more profound changes in collagen (and therefore dentine) properties immediately at the outset, reducing the potential for further change over time.

The loading rate and intervals may also play a role in strain development and relaxation. The present study loaded teeth at a maximum of every 15 mins using an irrigation regimen similar to Sobhani *et al.* (2010), whereas previous studies (Sim *et al.* 2001, Goldsmith *et al.* 2001, Rajasingham *et al.* 2010) loaded teeth following a 30 min irrigation period, as in group B. A longer interval between loading may have provided a safer interval for dentine to recover from accumulative loading stress but there did not appear to be a problem related to it as evidenced by figures 2~~1~~-~~4~~5 (Sim *et al.* 2001, Ng *et al.* 2020).

The most interesting outcomes in the present study are depicted in the raw data shown in figures ~~1~~2-~~4~~5; they confirm that individual differences between teeth may be the chief confounder in the lack of statistical convergence. Nevertheless, this data revealed that pre-load and loading TSS changes were unique for each tooth, which appeared to exhibit unique "strain limits" that could be reached either by irrigant introduction or tooth loading. Once reached, further change was restricted, unless, presumably, there was permanent structural change induced by thermal, chemical or mechanical means (Luescher *et al.* 1974, Stryer 1981, Osborn 1982, Wang *et al.* 2002, McGee *et al*. 2012, Bertinetti *et al.* 2015).

The present study is to our knowledge, the first to report mechanical changes in dentine measured as "strain-shift" *during the irrigation period*. The term "strain-shift" was coined to describe strain changes without applied loading because the strain appeared to "drift" but it was not a function of the measurement system, nor was it random; it appeared related to definite intra-canal events.

The "strain-shift" data reflected strain changes due to irrigation alone without any loading. The pre-load strain-shift (με) exerted a negative influence on loading TSS during irrigation, regardless of irrigant or temperature. Strain-shift data for saline at 21°C showed a significant increase in strain immediately following irrigant introduction but subsequent irrigation resulted in much smaller changes suggesting some sort of equilibrium or a maximum limit had been reached. Goldsmith *et al.* (2001) also reported this variability in strain recordings but had not measured pre-load strain changes. The strain-shift data for saline at 60°C followed a similar pattern but was of a greater magnitude resulting in smaller strain changes in later irrigation cycles, suggesting a greater proportion of the strain limit had already been "used-up". The observed effect may be explained by some form of chemical change in conformation of collagen-mineral interface that reaches saturation, after which further strain changes may be limited owing to a pre-existing strain-limit having been reached. The effect may be mediated by direct effect of the salt solution on collagen conformation (Luescher *et al.* 1974, McGee *et al*. 2012) or *via* osmotic alteration of water saturation of collagen (Bertinetti *et al.* 2015). The latter showed enormous stress and strain build-up in collagen and associated mineral as a function of dehydration mediated by osmotic pressure. Alternatively, the osmotic effect could also have acted on the unbound water in the dentinal tubules, possibly drawing it out (Kishen & Asundi 2005, Granke *et al*. 2015).

The observations in this study serve to confirm the "pre-stressed laminate" model of tooth structure. The proposed analogy is that of a tooth conceived of as a three-dimensional nano-chain-link structure, which upon having a force applied would distort in the forced direction, taking up any slack in the tooth "structure system". However, further distortion would be limited unless and until the chain-links were plastically distorted or broken. The chain links may be represented in the tooth structure by the interconnection between collagen and hydroxyapatite, as mediated by the presence or absence of bound water. Saline appeared to allow tension and strain to develop (Bertinetti *et al.* 2015), whilst NaOCl appeared to release the tension (presumably through breaking of links), relaxing the structure and preventing transmission of strain from the occlusal loading site to the cervical measurement site.

A change in temperature from either saline at 21°C to NaOCl at 60°C or from saline at 60°C to NaOCl at 21°C demonstrated substantial strain-shifts during each episode of irrigation with NaOCl (at either temperature) for the remainder of the experiment. The strain-shift after irrigation (and before loading) was far greater than any random pre-irrigation strain-shift. These observations are also explainable using the proposed model, through alterations in collagen (Miles & Ghelashvili 1999, Vangsness *et* *al.* 1997, Wang *et al.* 2002, Armstrong *et al*. 2008, Hayashi *et al.* 2012, Gevorkian *et al*. 2013, Suwa *et al*. 2016).

The effects of thermal expansion and contraction of dentine (Lin *et al.* 2010) and the influence of temperature on the strain gauge should be considered (Saw & Messer 1995, Shrestha *et al.* 2010). A temperature rise of 40°C (21°C to 60°C) would have resulted in an error of 0.4%, based on the gauge factor; since the microstrain output was of the order of several hundred, a 0.4% error, would not have had a large impact on the results.

The *rate* of strain-shift, although not measured, was observed to be faster with NaOCl irrigation compared to saline. It was observed that the rate of change with NaOCl at 60°C was far greater than at 21°C, although this was not evident when saline 60°C was used.

The findings of this study offer further and new insight about the behaviour of teeth under specific conditions of irrigant delivery at various temperatures and emphasises the importance of synthesis of data from dentine samples and "whole" tooth models. Whilst the findings do not directly infer increased susceptibility to fracture, they offer new insight about the biomechanics of teeth that may be affected by cyclic loading at low levels.

Conclusions

Tooth anatomy significantly affected its strain characteristics, exhibiting limits within which strain changes occurred. Intra-canal introduction of saline or NaOCl caused non-random strain shifts without loading. Non-random strain shifts during the non-loading phase were related to intra-canal irrigation, including an immediate impact upon first delivery of saline and also most deliveries of NaOCl. Pre-load strain changes negatively influenced loading strain with both irrigants. Irrigation with NaOCl-21°C increased loading tooth strain, as did saline-80°C or NaOCl-80°C but NaOCl-60°C decreased it. A "chain-link" model was proposed to explain the findings and tooth biomechanics.

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Tables

Table 1 Experimental and anatomical grouping with Irrigation and loading sequences

Group	Root type	Number of teeth	Irrigant sequence	Loading interval (minute)	Duration (minute)
A1	Single	6	NaOCl-21°C	15	120
A2	Single	6	NaOCl-60°C	15	120
A3*	Single	6	Saline-21°C	15	120
			NaOCl-21°C	15	120
A4*	Single	6	Saline-60°C	15	120
			NaOCl-21°C	15	120
A5*	Single	6	Saline-21°C	15	120
			NaOCl-60°C	15	120
A6*	Single	6	Saline-60°C	15	120
			NaOCl-60°C	15	120
B1	Single	5**	Saline-21°C	30	90
	Fused	5			
	Double	4			
B2	Single	5	Saline-80°C	30	90
	Fused	5**			
	Double	4			
B3	Single	5	NaOCl-21°C	30	90
	Fused	5			
	Double	4			
B4	Single	5	NaOCl-80°C	30	90
	Fused	5			
	Double	4			

*Irrigation with saline (21°C or 60°C) accompanied by loading at every 15 mins up to 120 mins; followed by NaOCl (21°C or 60°C) accompanied by loading at every 15 mins from 135-255 mins.

**One tooth lost through fracture in these groups.

Table 2. Mean and standard deviations (SD) of loading TSS (pe) at selected irrigation periods

For Groups A1-6 in single-rooted teeth, irrigation periods (IP) shown are: 0 (0 mins), 1 (12 mins), 9 (132 mins), 10 (147 mins) and 18 (267 mins); for Groups B1-4 including mixed root anatomy teeth, IP shown are: 0 (0 min), 1 (30 mins), 2 (60 mins), and 3 (90 mins).

Groups	Irrigation stage	Mean (pe)	SD (pe)
A1 (n=6)	IP 0 (Baseline)	8.19E+2	6.61E+2
	IP 1 (NaOCl 21°C)	8.26E+2	6.58E+2
	IP 9 (NaOCl 21°C)	8.70E+2	7.71E+2
A2 (n=6)	IP 0 (Baseline)	3.81E+2	1.21E+2
	IP 1 (NaOCl 60°C)	3.94E+2	1.50E+2
	IP 9 (NaOCl 60°C)	3.56E+2	1.24E+2
A3 (n=6)	IP 0 (Baseline)	2.32E+2	1.31E+2
	IP 1 (Saline 21°C)	2.58E+2	1.12E+2
	IP 9 (Saline 21°C)	2.30E+2	1.39E+2
	IP 10 (NaOCl 21°C)	2.39E+2	1.42E+2
	IP 18 (NaOCl 21°C)	2.30E+2	1.73E+2
A4 (n=6)	IP 0 (Baseline)	3.78E+2	3.28E+2
	IP 1 (Saline 60°C)	3.82E+2	3.65E+2
	IP 9 (Saline 60°C)	3.73E+2	3.38E+2
	IP 10 (NaOCl 21°C)	3.68E+2	3.37E+2
	IP 18 (NaOCl 21°C)	4.43E+2	4.39E+2
A5 (n=6)	IP 0 (Baseline)	3.24E+2	2.34E+2
	IP 1 (Saline 21°C)	3.08E+2	2.31E+2
	IP 9 (Saline 21°C)	3.41E+2	2.36E+2
	IP 10 (NaOCl 60°C)	3.50E+2	2.31E+2
	IP 18 (NaOCl 60°C)	2.51E+2	1.18E+2
A6 (n=6)	IP 0 (Baseline)	3.87E+2	1.60E+2
	IP 1 (Saline 60°C)	3.86E+2	1.85E+2
	IP 9 (Saline 60°C)	4.08E+2	1.73E+2
	IP 10 (NaOCl 60°C)	4.33E+2	1.80E+2
	IP 18 (NaOCl 60°C)	4.31E+2	1.89E+2
B1 (n=12)	IP 0 (Baseline)	3.95E+3	1.85E+3
	IP 1 (Saline 21°C)	3.96E+3	1.77E+3
	IP 2 (Saline 21°C)	3.92E+3	1.71E+3
	IP 3 (Saline 21°C)	3.89E+3	1.72E+3
B2 (n=13)	IP 0 (Baseline)	2.99E+3	1.72E+3
	IP 1 (Saline 80°C)	3.21E+3	1.83E+3
	IP 2 (Saline 80°C)	3.18E+3	1.55E+3
	IP 3 (Saline 80°C)	3.45E+3	1.64E+3
B3 (n=14)	IP 0 (Baseline)	3.67E+3	2.11E+3
	IP 1 (NaOCl 21°C)	3.72E+3	2.20E+3
	IP 2 (NaOCl 21°C)	3.65E+3	2.22E+3
	IP 3 (NaOCl 21°C)	3.66E+3	2.29E+3
B4 (n=14)	IP 0 (Baseline)	3.73E+3	2.44E+3
	IP 1 (NaOCl 80°C)	3.85E+3	2.33E+3
	IP 2 (NaOCl 80°C)	4.04E+3	2.30E+3
	IP 3 (NaOCl 80°C)	3.85E+3	2.17E+3

For groups A1-6, loading data were recorded every 15 minutes during the irrigation phase (total of 9 points per A1/A2 samples and 18 points per A3-A6 samples), however only those at baseline, at the end of first cycle, and at the end of all irrigation cycles for each test irrigant were selected for analyses.

For groups B1-3, loading data were recorded at baseline and at every 30 minutes during the irrigation phase with a total of 4 data points per sample.

Table 3. Linear mixed models investigating the effect of time on loading TSS (pe) over the entire course of irrigation with saline at 21°C or 60°C, or NaOCl at 21°C or 60ºC, of teeth from groups A1-6
(all relevant data were included for analysis in each model)

	Coefficient	95% CI for coefficient	*P* value
Model 1 incorporating data from Saline-21°C irrigation: groups A3,5 (n=12)			
Time	-0.02	-0.15, 0.11	0.7
Random effect parameters	Estimate	92% CI for Estimate	
Variance for each tooth	32323.6	14508.8, 72012.5	
Variance for each observation	323.3	243.6, 429.0	
Model 2 incorporating data from Saline-60°C irrigation: groups A4,6 (n=12)			
Time	0.06	-0.07, 0.19	0.3
Random effect parameters	Estimate	92% CI for Estimate	
Variance for each tooth	62185.5	27925.0, 138479.3	
Variance for each observation	312.5	235.5, 414.7	
Model 3 incorporating data from NaOCl-21°C irrigation: groups A1,3,4 (n=18)			
Time	0.55	0.12, 0.98	0.01
Pre-irrigation			
None (Reference)			
Saline 21°C	-575.2	-1069.5, -80.9	0.02
Saline 60°C	-417.3	-911.6, 77.0	0.1
Random effect parameters	Estimate	92% CI for Estimate	
Variance for each tooth	190226.9	98781.8, 366325.1	
Variance for each observation	5193.1	4122.1, 6542.5	
Model 4 incorporating data from NaOCl-60°C irrigation: groups A2, 5, 6 (n=18)			
Time	-0.43	-0.69, -0.17	0.001
Pre-irrigation			
None (Reference)			
Saline 21°C	-56.9	-238.4, 124.6	0.5
Saline 60°C	80.2	-101.3, 261.7	0.4
Random effect parameters	Estimate	92% CI for Estimate	
Variance for each tooth	25509.4	13202.1, 49289.7	
Variance for each observation	1878.2	1490.8, 2366.2	

Table 4. Linear mixed models investigating the effect of pre-load strain shift (pe) on the corresponding TSS by type and temperature of irrigant, of teeth from groups A1-6 (all relevant data were included for analysis in each model)

	Coefficient	95% CI for coefficient	*P* value
Model 1 Saline-21°C (n=6 teeth)			
Pre-load strain shift	-0.03	-0.05, -0.01	0.001
Random effect parameters	Estimate	92% CI for Estimate	
Variance for each tooth	32502.4	14590.4, 72403.8	
Variance for each observation	288.2	217.2, 382.5	
Model 2 Saline-60°C (n=6 teeth)			
Pre-load strain shift	-0.006	-0.014, 0.002	0.2
Random effect parameters	Estimate	92% CI for Estimate	
Variance for each tooth	62049.3	27863.8, 138176.4	
Variance for each observation	309.5	233.2, 410.7	
Model 3 NaOCl-21°C (n=6 teeth)			
Pre-load strain shift	-0.05	-0.12, 0.02	0.1
Random effect parameters	Estimate	92% CI for Estimate	
Variance for each tooth	73432.8	32829.5, 164254.2	
Variance for each observation	3446.1	2596.9, 4573.0	
Model 4 NaOCl-60°C (n=6 teeth)			
Pre-load strain shift	-0.04	-0.11, 0.02	0.2
Random effect parameters	Estimate	92% CI for Estimate	
Variance for each tooth	35427.3	15805.5, 79408.6	
Variance for each observation	2558.3	1927.8, 3394.8	

Table 5. Linear mixed models investigating the effect of time on loading TSS (pe) over the course of irrigation with saline or NaOCl, at 21°C or 80ºC, respectively, of teeth from groups B1-4

	Coefficient	95% CI for coefficient	*P* value
Model 1 incorporating data from Saline-21°C irrigation: group B1 (n=13)			
Time	-0.72	-2.74, 1.30	0.5
Root			
Single (Reference)	-		
Double	-2222.75	-4346.52, -98.98	0.04
Fused	-218.57	-2233.35, 1796.21	0.8
Mesio-distal thickness	-2366.06	-5042.8, 310.7	0.08
Random effect parameters	Estimate	95% CI for Estimate	
Variance for each tooth	2.3E+6	1.1E+6, 5.1E+6	
Variance for each observation	6.2E+4	4.0E+4, 9.7E+4	
Model 2 incorporating data from Saline-80°C irrigation: group B2 (n=13)			
Time	4.58	1.39, 7.76	0.005
Root			
Single (Reference)	-		
Double	-1523.50	-3383.55, 336.55	0.1
Fused	93.83	-1766.22, 1953.88	0.9
Mesio-distal thickness	-2252.50	-4150.32, -354.67	0.02
Random effect parameters	Estimate	92% CI for Estimate	
Variance for each tooth	1.9E+6	9.0E+5, 4.3E+6	
Variance for each observation	1.5E+5	9.9E+4, 2.4E+5	
Model 3 incorporating data from NaOCl-21°C irrigation: group B3 (n=14)			
Time	-0.34	-2.63, 19.6	0.7
Root			
Single (Reference)	-		
Double	-2723.36	-5086.18, -360.53	0.02
Fused	-1703.05	-3930.74, 524.64	0.1
Mesio-distal thickness	-3198.27	-6318.34, -78.20	0.045
Random effect parameters	Estimate	95% CI for Estimate	
Variance for each tooth	3.0E+6	1.5E+6, 6.8E+6	
Variance for each observation	8.4E+4	5.6E+4, 1.3E+5	
Model 4 incorporating data from NaOCl-80°C irrigation: group B4 (n=14)			
Time	1.86	-1.27, 5.00	0.2
Root			
Single (Reference)	-		
Double	-2915.89	-5379.87, -451.91	0.02
Fused	-1479.71	-3802.77, 843.35	0.2
Mesio-distal thickness	-2107.11	-5701.88, 1487.65	0.3
Random effect parameters	Estimate	92% CI for Estimate	
Variance for each tooth	4.4E+6	2.1E+6, 9.3E+6	
Variance for each observation	1.6E+5	1.1E+5, 2.5E+5	

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CRediT author statement

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Kishor Gulabivala - Conceptualization, Resources, Methodology, Supervision, writing, analysis.

Imran Azam – Investigation, data curation, visualisation, writing, analysis.

Shima Mahdavi-Izadi – Investigation, data curation, visualisation, writing, analysis.

Graham Palmer - Methodology, Supervision, editing.

George Georgiou - Methodology, Supervision, editing.

Jonathan Knowles – Conceptualisation, Resources, Methodology, Supervision, analysis.

Yuan Ling Ng - Conceptualization, Methodology, Supervision, visualisation, analysis.

Figures

Figure 1 Pictogram depicting the methodology

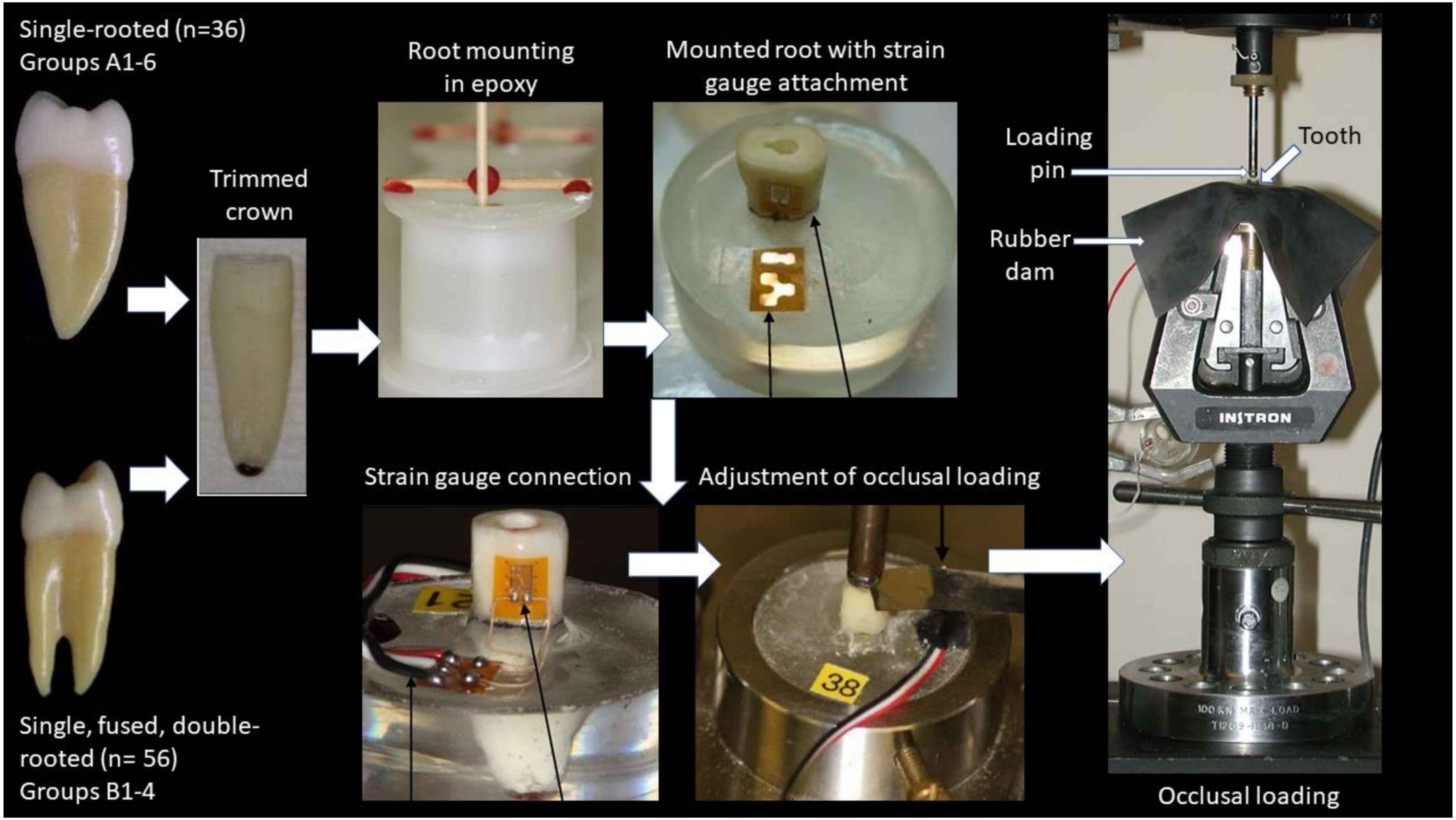


Figure ~~1~~2 Scatter plot of TSS over time for Group A3 (Saline-21°C then NaOCl-21°C)
(Hollow markers represent preload-TSS; solid markers represent loading-TSS)

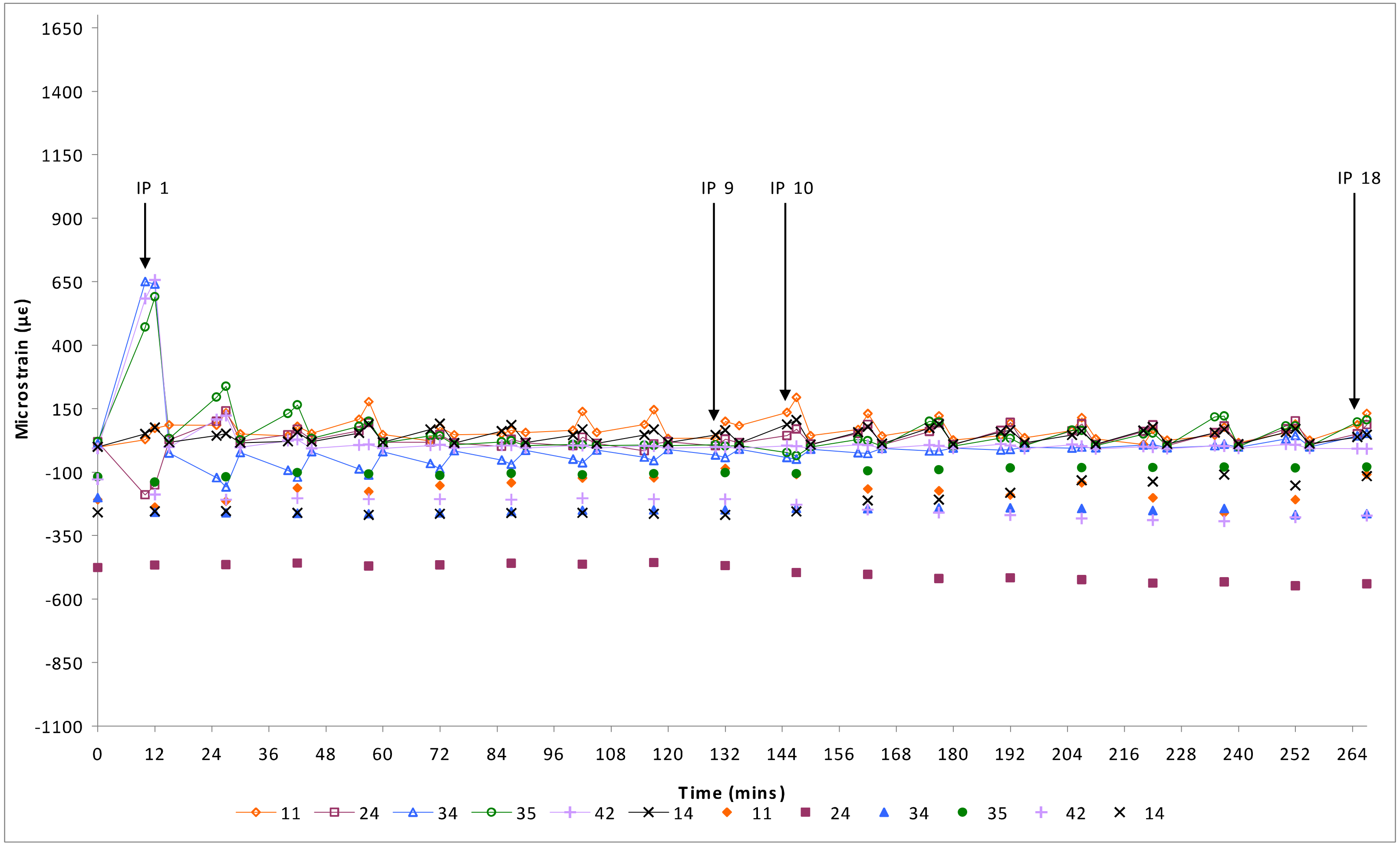


Figure ~~2~~3 Scatter plot of TSS over time for Group A4 (Saline-60°C then NaOCl-21°C)
(Hollow markers represent preload-TSS, solid markers represent loading-TSS)

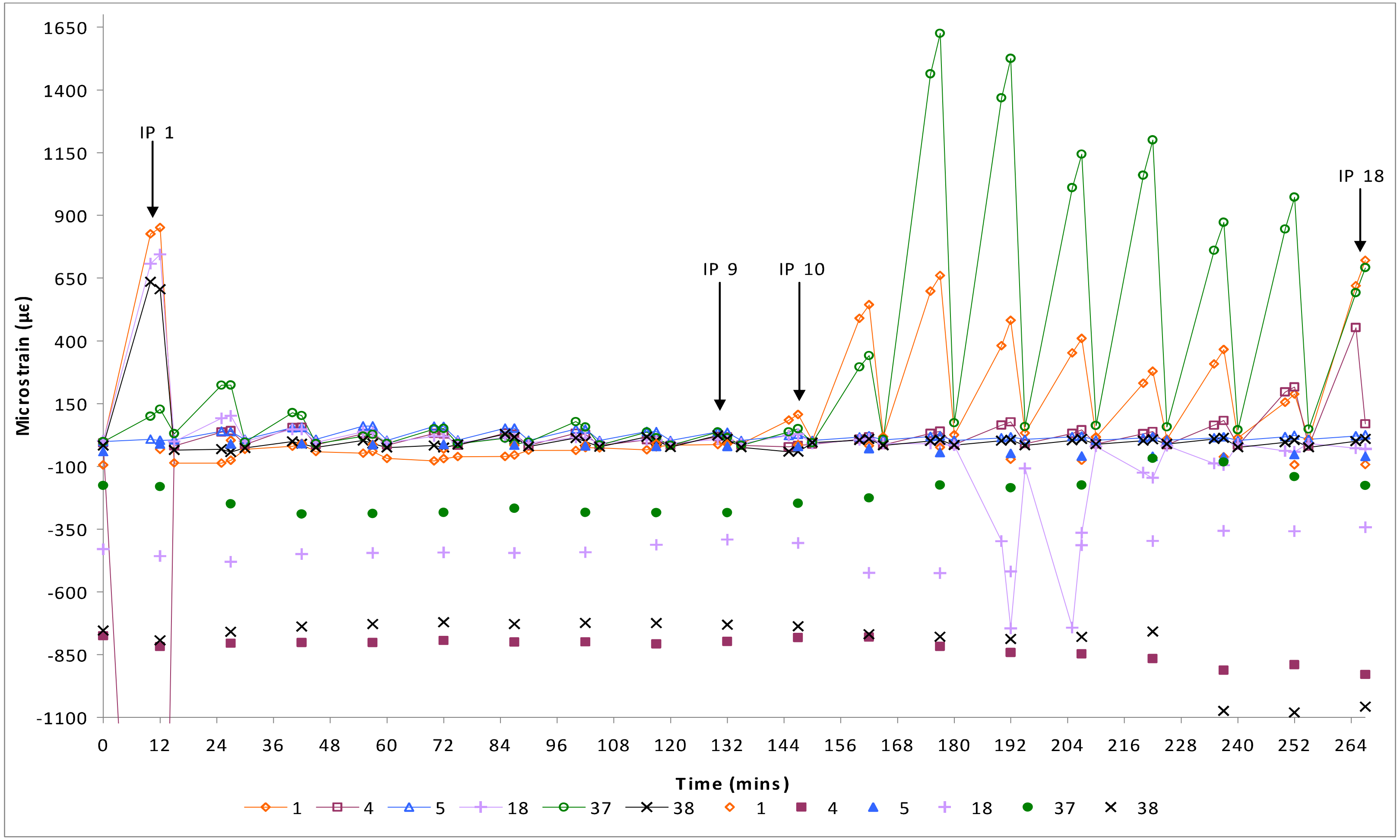


Figure ~~3~~4 Scatter plot of TSS over time for Group A5 (Saline-21°C then NaOCl-60°C)
(Hollow markers represent preload-TSS, solid markers represent loading-TSS)

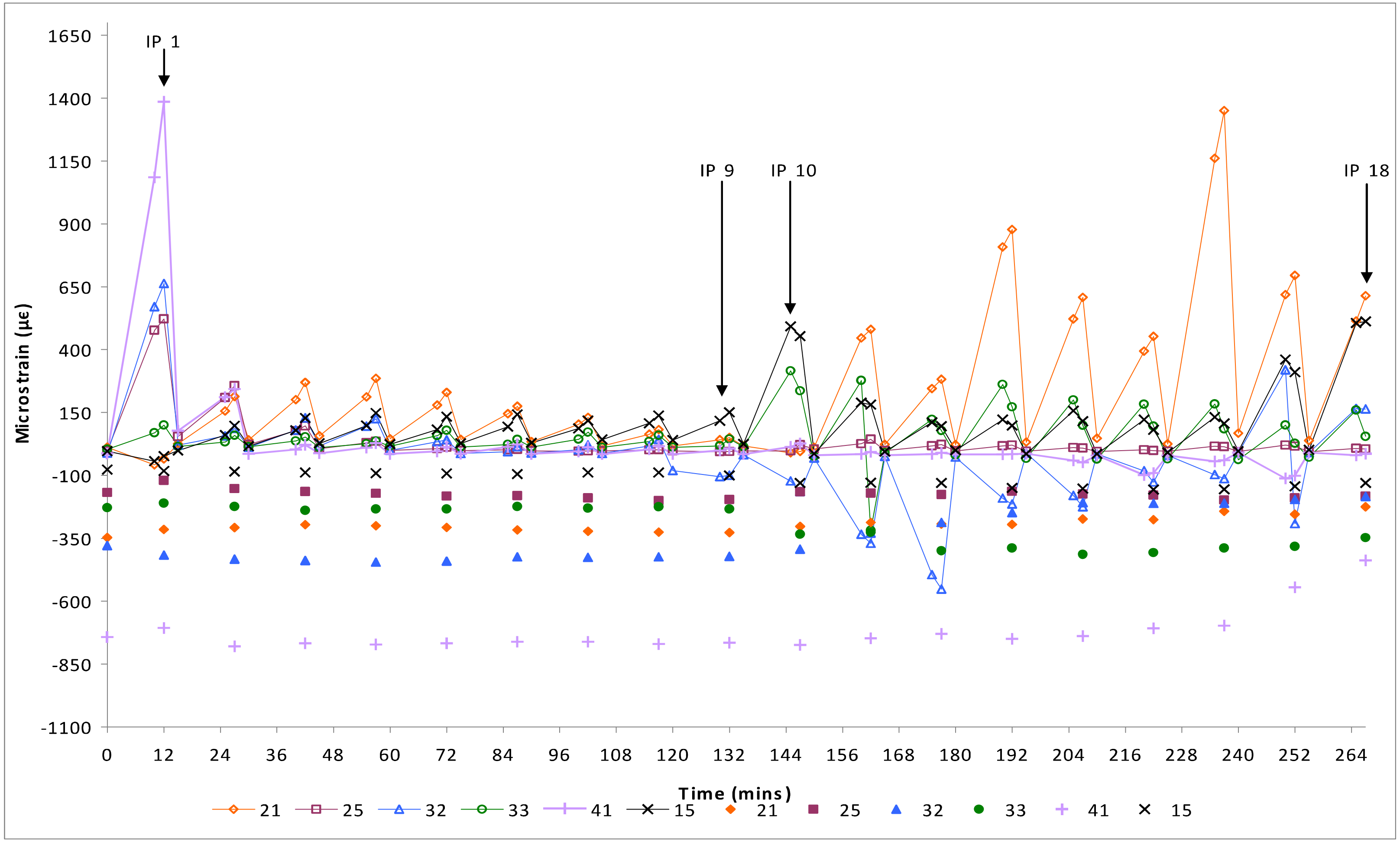


Figure 45 Scatter plot of TSS over time for Group A6 (Saline-60°C then NaOCl-60°C)
(Hollow markers represent preload-TSS, solid markers represent loading-TSS)

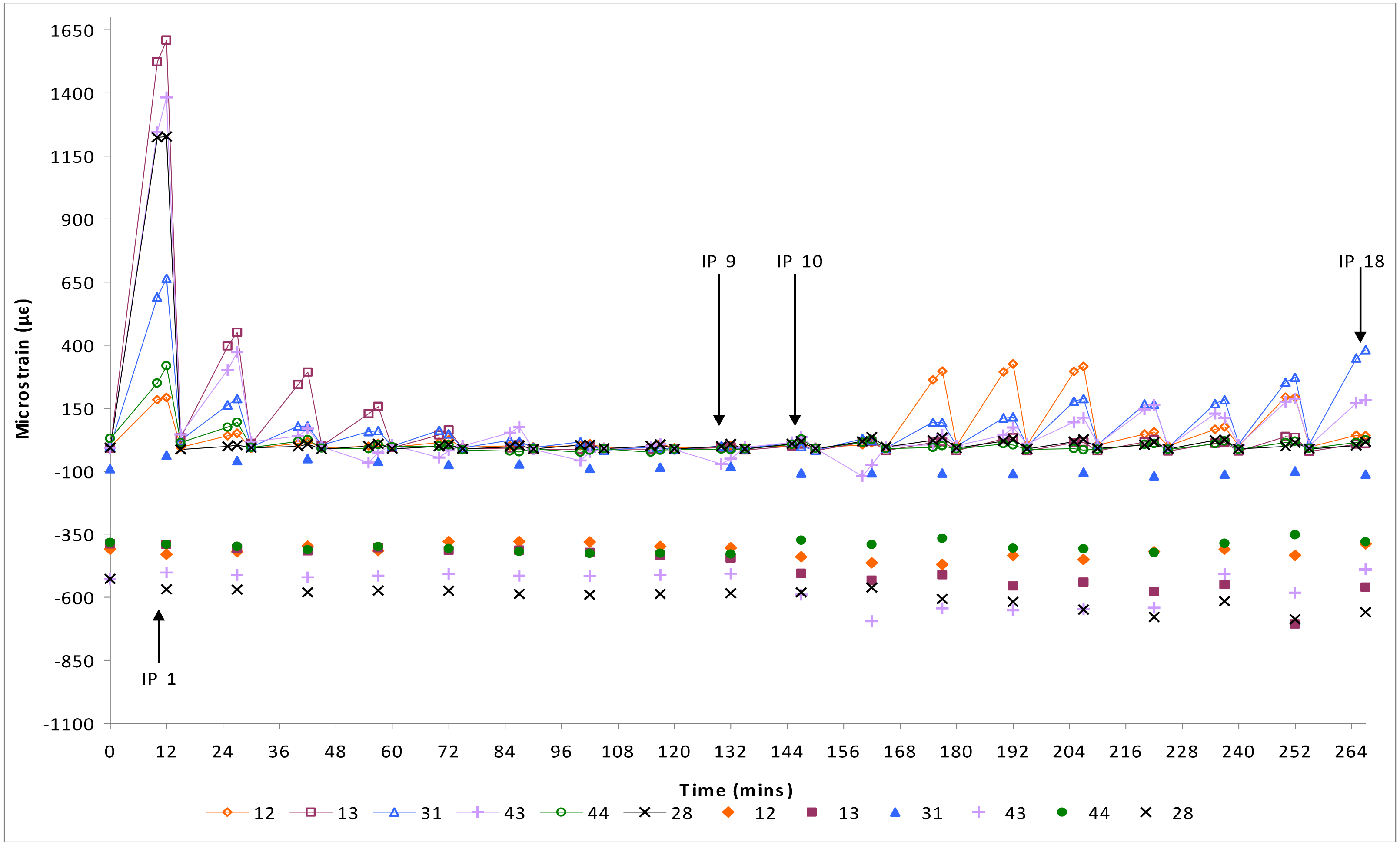


Figure ~~5~~6 Scatter plot of TSS over time for Group B1 (Saline-21°C)
(▲ = single-rooted teeth, ◆ = Fused-rooted teeth, ■ = Double-rooted teeth)

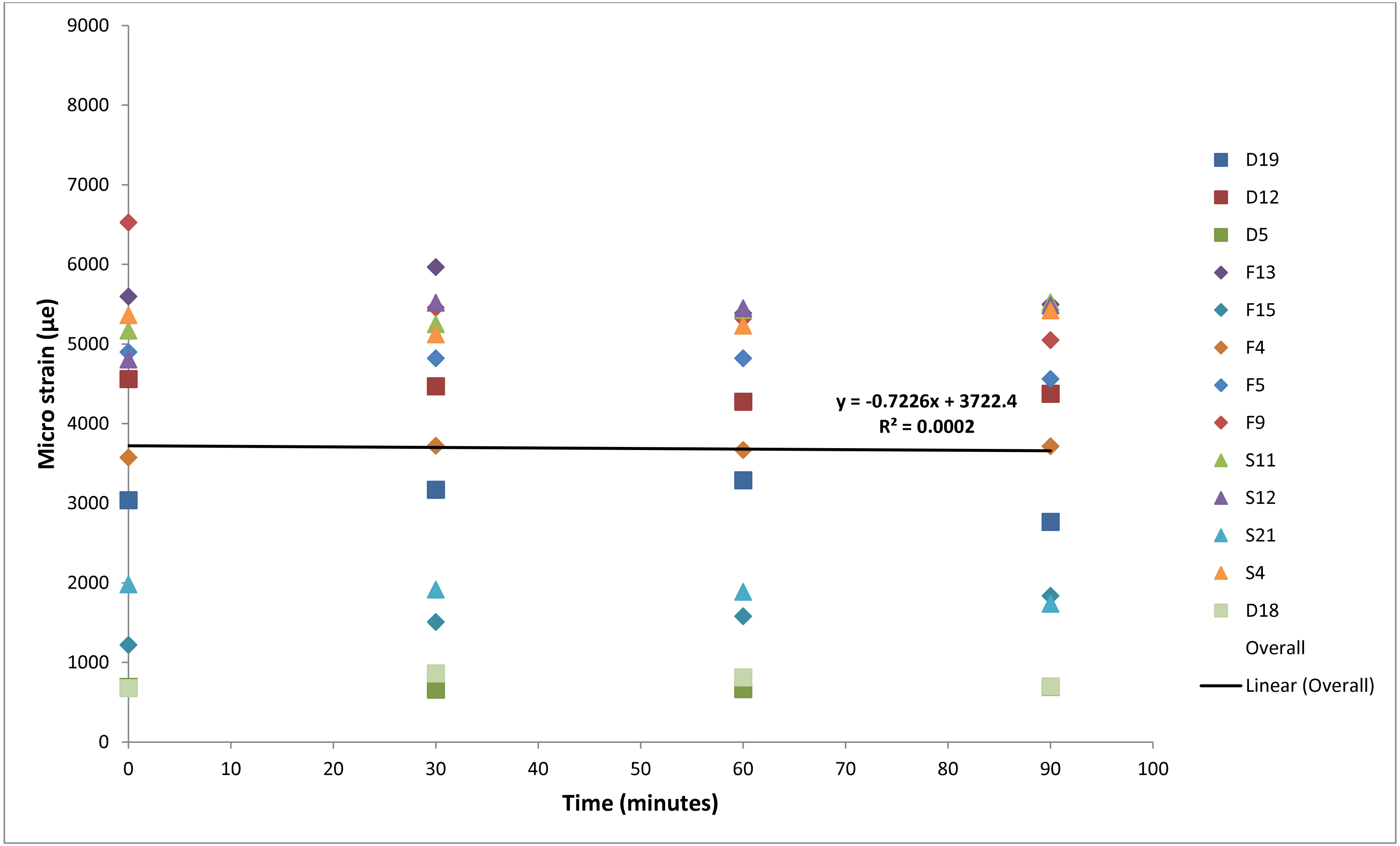


Figure ~~6~~7 Scatter plot of TSS over time for Group B2 (Saline-80°C)
(▲ = single-rooted teeth, ◆ = Fused-rooted teeth, ■ = Double-rooted teeth)

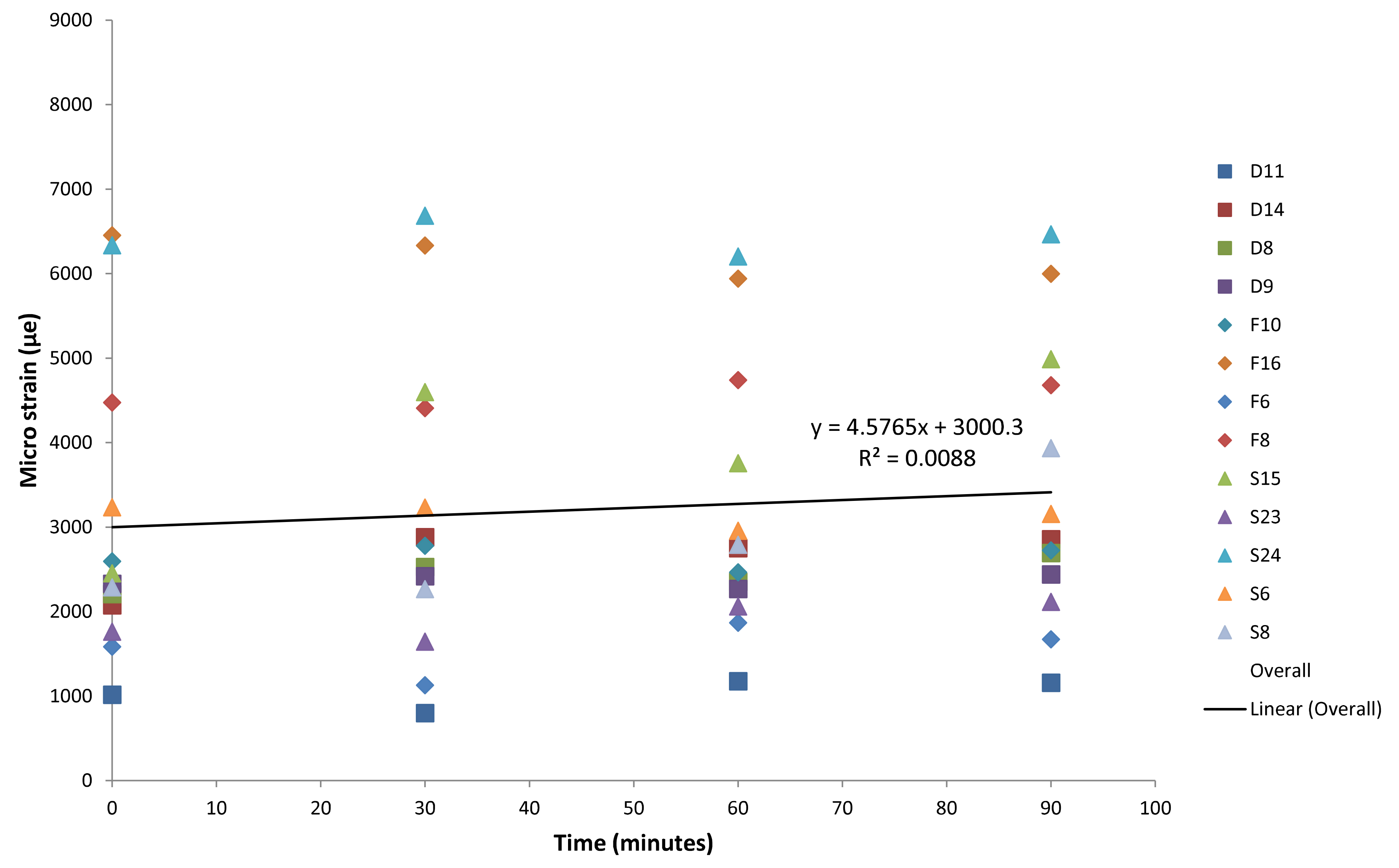


Figure ~~7~~8 Scatter plot of TSS over time for Group B3 (NaOCl-21°C)
(▲ = single-rooted teeth, ◆ = Fused-rooted teeth, ■ = Double-rooted teeth)

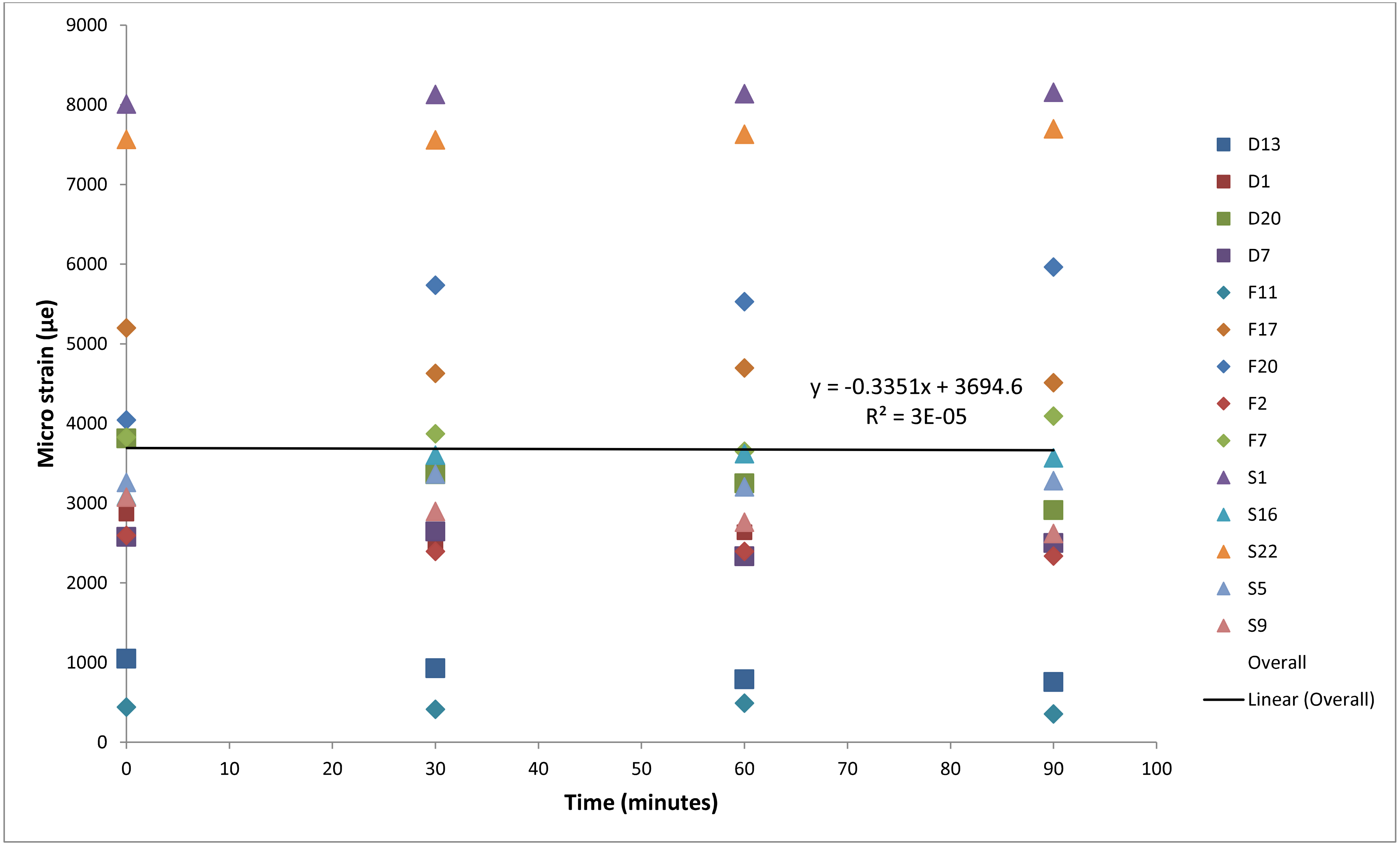
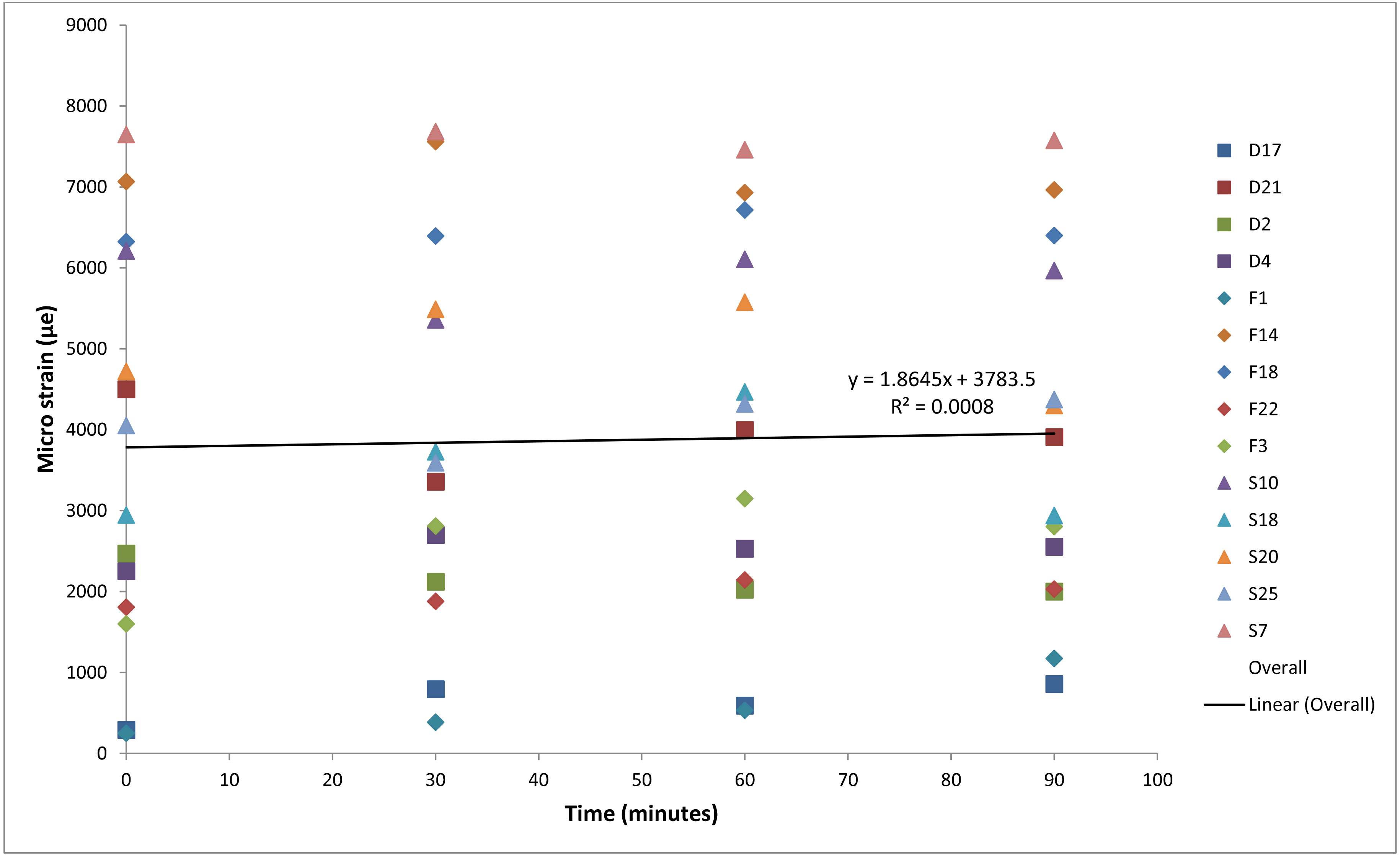


Figure 89 Scatter plot of TSS over time for Group B4 (NaOCl-80°C)
(▲ = single-rooted teeth, ◆ = Fused-rooted teeth, ■ = Double-rooted teeth)



Revised figure 1