

Journal of Endodontics

Effect of heated sodium hypochlorite on the viscoelastic properties of dentin evaluated using Dynamic Mechanical Analysis

--Manuscript Draft--

Manuscript Number:	JOE 19-128R2
Article Type:	Basic Research - Technology
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Manuscript Region of Origin:	Europe & Central Asia
Abstract:	<p>Introduction: This study aimed to investigate the effect of immersion of standardised dentin bars in 5% sodium hypochlorite (NaOCl) solutions at 60°C and 80°C on their viscoelastic properties, using Dynamic Mechanical Analysis (DMA).</p> <p>Methods: Eighteen intact teeth were used to produce ninety-nine dentin bars of standard dimensions (12mm×1mm×2mm) and randomly allocated to 6 groups (n=15 each) for immersion in: (1) Saline at 26°C; (2) Saline at 60°C; (3) Saline at 80°C; (4) NaOCl at 26°C; (5) NaOCl at 60°C; (6) NaOCl at 80°C. The bars were individually tested using DMA at baseline and after every 10 minutes of immersion in the test medium, up to 40 minutes. The effects of media, temperature, duration of exposure, and aspect ratio of bars on storage modulus (SM) and tan delta (TD) were investigated using Generalised Estimating Equations.</p> <p>Results: There was significant interaction between test medium and duration of immersion ($p<0.05$). The SM of specimens immersed in NaOCl at 60°C or 80°C decreased significantly ($p<0.0001$) over time of exposure, but the changes in other groups were minimal and insignificant. The TD of specimens immersed in saline 80°C ($p<0.05$), NaOCl at 60°C ($p<0.05$) or 80°C ($p<0.0001$) increased significantly over time of exposure but the change in NaOCl at 26°C was minimal. Other groups displayed negligible changes.</p> <p>Conclusions: NaOCl at 60°C or 80°C significantly reduced the elastic behaviour but increased the hysteresis of dentin under cyclic loading.</p>

Effect of heated sodium hypochlorite on the viscoelastic properties of dentin evaluated using Dynamic Mechanical Analysis

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47 Acknowledgments

48 The authors affirm that there was and is no financial affiliation (e.g., employment, direct payment, stock
49 holdings, retainers, consultantships, patent licensing arrangements or honoraria), or involvement with any
50 commercial organization with direct financial interest in the subject or materials discussed in this
51 manuscript, nor have any such arrangements existed. The authors deny any other conflicts of interest
52 related to this study.
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Statement of clinical relevance

- Exposure of dentin to 5% NaOCl solutions heated to 60°C-80°C, over 40 minutes, reduced its elasticity and increased its viscous behaviour or hysteresis. These changes could potentially increase fracture susceptibility, particularly on sustained loading.

Highlights

- Non-destructive dynamic mechanical analysis allows investigation of the viscoelastic properties of dentin specimens within their elastic limit by cyclic loading.
- Immersion of dentin in 5% NaOCl solutions heated to 60°C or 80°C, over a 40-minute period, altered its viscoelastic properties by reducing elasticity (storage modulus) and increasing viscous behaviour ($\tan \delta$) with time.

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Abstract

Introduction: This study aimed to investigate the effect of immersion of standardised dentin bars in 5% sodium hypochlorite (NaOCl) solutions at 60°C and 80°C on their viscoelastic properties, using Dynamic Mechanical Analysis (DMA).

Methods: Eighteen intact teeth were used to produce ninety-nine dentin bars of standard dimensions (12mm×1mm×2mm) and randomly allocated to 6 groups (n=15 each) for immersion in: (1) Saline at 26°C; (2) Saline at 60°C; (3) Saline at 80°C; (4) NaOCl at 26°C; (5) NaOCl at 60°C; (6) NaOCl at 80°C. The bars were individually tested using DMA at baseline and after every 10 minutes of immersion in the test medium, up to 40 minutes. The effects of media, temperature, duration of exposure, and aspect ratio of bars on storage modulus (SM) and tan delta (TD) were investigated using Generalised Estimating Equations.

Results: There was significant interaction between test medium and duration of immersion ($p<0.05$). The SM of specimens immersed in NaOCl at 60°C or 80°C decreased significantly ($p<0.0001$) over time of exposure, but the changes in other groups were minimal and insignificant. The TD of specimens immersed in saline 80°C ($p<0.05$), NaOCl at 60°C ($p<0.05$) or 80°C ($p<0.0001$) increased significantly over time of exposure but the change in NaOCl at 26°C was minimal. Other groups displayed negligible changes.

Conclusions: NaOCl at 60°C or 80°C significantly reduced the elastic behaviour but increased the hysteresis of dentin under cyclic loading.

Key words: Heated NaOCl, storage modulus, tan delta.

Introduction

Sodium hypochlorite (NaOCl) is the preferred root canal irrigant because of its antimicrobial (1) and tissue-dissolving (vital and necrotic) properties (2, 3). However, NaOCl also unfavourably alters flexural strength and elastic modulus of dentine (4, 5) and surface strain in “whole teeth” (6). The altered strain behaviour of teeth is worse when 5% NaOCl and 17% EDTA are used in alternation (6). This “whole tooth” strain behaviour is not entirely explained by quasi-static properties of dentin bars (4, 7). Non-destructive dynamic loading of dentin bars may better explain whole-tooth strain behaviour.

The changes in mechanical properties of dentin are attributed to depletion of dentinal collagen by NaOCl and mineral by EDTA (8, 9). Type I collagen accounts for 22% organic material by weight and contributes considerably to the mechanical properties of dentin (10).

The dissolution effect of NaOCl on pulp collagen increases with temperature because of an increased rate of reaction, prompting the adoption of heated solutions in dental practice (11). Collagen consisting of 3 helical polypeptide chains bound together by hydrogen and covalent bonds into a super-helix (12), undergoes various temperature-dependent and time-dependent changes (13). Collagen structure is altered to different degrees at different temperatures (20-200°C) and is influenced by levels of hydration and physical confinement in mineralized tissues (12-16).

Given the avocation of using heated NaOCl to 60°C (<https://www.endoruddle.com/blogs/show/15/heating-sodium-hypochlorite-peer-reviewed-evidence-supporting-the-heating-of-naocl>, accessed 13/03/2019) and its testing at such temperatures (11) and beyond (17, 18), it would be logical to investigate the effect of heated sodium hypochlorite on the viscoelastic properties of dentin under dynamic loading (19). We hypothesized that exposure of dentin bars to heated NaOCl would significantly change their *viscoelastic* properties. The aim of this study was to investigate the effect of immersion of standardized dentin bars in 5%

1 NaOCl solutions at 60°C or 80°C over a period of 40 minutes, on their viscoelastic
2 properties.
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5 **Materials and Methods**

6 ***Preparation of dentin bars***

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8 Approval was granted for the use of extracted teeth from the UCL Eastman Biobank (Study
9 number: 1301). Forty-one recently extracted wisdom teeth were stored in 4% formal-saline
10 for 2 to 12 weeks before commencement of experiment. Eighteen intact, disease- and crack-
11 free teeth (from patients aged 18-35 years) with fused roots of at least 13 mm length, were
12 selected. Adherent soft or hard tissues were removed gently from the teeth with a scalpel.
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22 The teeth were sectioned longitudinally in the mesio-distal plane and slices were produced in
23 the bucco-lingual plane using a revolving diamond-coated saw (Leica Instruments Gmbtt
24 Mod. 1.600, D-6907 Nussloch, Germany). Dentin bars, of standard dimensions (12 mm × 1
25 mm × 2 mm) were produced under copious water irrigation and their length and width
26 measured with a micrometer (Moore and Wright, Bradford, UK) and recorded (Pyriss
27 software, Perkin Elmer instruments, Beaconsfield, UK). Each tooth produced two to six bars,
28 mainly from the root, yielding a total of ninety-nine bars; all specimens were marked with an
29 indelible pen to identify the surface facing the canal lumen and stored in 0.9% saline solution
30 (Seven Biotech Ltd., Kidderminster, UK). The dentinal tubules ran across the bar so that
31 loading was parallel to the dentinal tubules.
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45 ***Allocation of dentin bars to test groups***

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47 Ninety dentin bars were randomly assigned to six groups (n=15), three were used for a pilot
48 study (Table 1) and six were randomly pre-assigned as 'spares' in case of need for
49 replacement. All dentin bars were stored in saline just prior to the experiment.
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Preparation of sodium hypochlorite (5%) test solution

Stock sodium hypochlorite solution (6-14% active chlorine) (Merck KGaA, Darmstadt, Germany) was diluted with distilled water to produce 5% NaOCl, confirmed by iodometric titration, just before use.

The test solutions (40 mL) were decanted into individual 120 mL glass jars and heated to the designated temperatures (60°C and 80°C) over a hot-plate (Jenway 1000, Chelmsford, Essex, UK). The temperature was monitored with a thermometer (Fisherbrand, Thermo Fisher Scientific Inc., Loughborough, UK) throughout the experiment. The glass jar remained on the hot plate, covered with a lid during the immersion period, as the solution was constantly stirred. Fresh solution was prepared and heated for each bar.

Dynamic mechanical analysis (DMA) test conditions

The viscoelastic properties of dentin were evaluated at baseline and after every 10 minutes of immersion in the test medium, up to 40 minutes, using the 3-point bend test in a DMA-7e (Perkin Elmer instruments, Beaconsfield, UK) with Pyris software (Perkin Elmer instruments, Beaconsfield, UK). The loss and storage moduli and $\tan \delta$ were recorded. The specimens were subjected to oscillatory and static deformation. A static tension control of 120% (i.e. dynamic stress $\times 1.2 =$ static stress) and a constant dynamic strain control of 0.02% (deformation over length of specimen) were maintained. The initial loads applied were 200 mN and 240 mN for dynamic and static force, respectively; these parameters adjust accordingly, but at the same ratio with respect to each other, so as to satisfy the dynamic strain control conditions throughout testing. The frequency of loading was held at 1 Hz. All tests were performed at room temperature (26°C).

Upon removal from the storage solution, a test run was performed before immersion in its respective test solution. The duration of each run was one minute and the readings for storage modulus, loss modulus and $\tan \delta$ were taken at 30 seconds into each run. The total

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time to complete each test, including the time to equilibrate the apparatus (for each test) and establish a stable dynamic strain was approximately 7 minutes.

The specimens were then immersed in their respective test solutions for 10 minutes and then quenched by rinsing in saline for three minutes before the test. The bars were left damp during the test and their orientation maintained constant with the marked side under compression so that the dentinal tubules had a consistent orientation to the probe. The cycle of immersion, wash and test-run was repeated 4 times until a total of 40 minutes exposure to the test medium was completed for each sample.

Data analysis

The data for storage modulus, loss modulus and $\tan \delta$ were recorded and stored on Pyris Data Analysis software and analyzed using the SPSS statistical software package (SPSS Inc., Chicago, IL, USA). The means and 95% confidence intervals of storage modulus (SM) and $\tan \delta$ were plotted against the duration of immersion by each group. Generalized Estimating Equations (GEE) were used to investigate the effects of test medium and duration of immersion on SM and $\tan \delta$, accounting for the effects of specimen dimensions.

Results

It was observed that the bars in groups with heated NaOCl at 60°C and 80°C effervesced during immersion in the test solution, gaining a chalky appearance; those in the latter group being more obvious.

The mean values and 95% confidence intervals for SM and $\tan \delta$ at baseline and following each 10-minute immersion for all test and control groups are presented in figure 1. The mean SM at baseline ($t = 0$ minute) varied between 14.35 GPa (95% confidence interval [CI]: 12.41 – 16.29 GPa) and 19.92 GPa (95% CI: 17.25 – 22.59), while the mean $\tan \delta$ at

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2 baseline (t = 0 minute) varied between 0.077 (95% CI: 0.069 - 0.085) and 0.098 (95% CI:
3 0.089 - 0.108) amongst the test groups, respectively.
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5 In groups with NaOCl 60°C and 80°C, a clear downward trend was evident in the storage
6 modulus values (reduced by 5.6 [95% CI: 4.3, 6.8] GPa, 7.4 [95% CI: 5.1, 9.7] GPa,
7 respectively) with time but an upward trend in the tan δ values (increased by 0.03 [95% CI:
8 0.02, 0.05] and 0.07 [95% CI: 0.03, 0.10] units, respectively).
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14 The GEE models revealed test medium and duration of immersion had significant
15 (p<0.0001) interactive effects on SM and tan δ of the dentin bars, respectively (Tables 2&3).
16 This indicated that the duration of immersion had different effects among different test
17 media. Every minute of immersion in NaOCl at 60°C or 80°C resulted in 0.014 Pa
18 (p<0.0001) or 0.019 Pa (p<0.0001) reduction in SM, respectively. Concomitantly, there was
19 an increase in tan δ by 0.001 or 0.002 units for every minute of immersion in NaOCl at 60°C
20 (p<0.01) or 80°C (p<0.0001), respectively. Specimens immersed in saline at 80°C (p<0.05)
21 also displayed significant increase in tan δ , albeit small in magnitude.
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33 The reduction of SM (p<0.0001) and increase in tan δ (p<0.05) amongst samples in the
34 heated NaOCl groups was significantly greater than those in the heated Saline groups,
35 regardless of temperature.
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44 **Discussion**

45 The aspect ratio of the dentin bars can influence outcomes and thus every effort was made
46 to minimize sample deviation. The potential effect of aspect ratios (ratio of the span between
47 the supports for the specimen and the height) was accounted for in the regression models
48 and found to be statistically insignificant (p>0.05).
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56 The potentiating effect of heat on the antibacterial and tissue-dissolving properties of sodium
57 hypochlorite has been clinically applied using solution temperatures of up to 60°C
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2 [https://www.endoruddle.com/blogs/show/15/heating-sodium-hypochlorite-peer-reviewed-evidence-supporting-the-](https://www.endoruddle.com/blogs/show/15/heating-sodium-hypochlorite-peer-reviewed-evidence-supporting-the-heating-of-naocl)
3 [heating-of-naocl](https://www.endoruddle.com/blogs/show/15/heating-sodium-hypochlorite-peer-reviewed-evidence-supporting-the-heating-of-naocl), accessed 13/03/2019). Previous studies had tested NaOCl at 21°C to 60°C
4 (11); the upper limit temperature in this study (80°C) was used as a positive control because
5 the tri-helical structure of collagen denatures between 65°C – 80°C in a specific
6 temperature-dependent manner (12, 13, 20).
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12 Storage of teeth in formal-saline (4%) was found to have no effect on the viscoelastic
13 properties of dentin harvested in a pilot study, even though it is known that it can induce
14 collagen cross-links that may affect the denaturation temperature of extracted native
15 collagen (21). Other forms of storage, such as freezing, may also induce conformational and
16 cross-linking changes in collagen but may affect the thermodynamic properties of collagen to
17 a greater degree (22).
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27 The overall duration of test was based on the average root canal treatment procedure
28 duration plus pilot studies, which showed that viscoelastic properties of dentin reached a
29 plateau between 40-60 minutes. Every effort was made to reposition the bars accurately on
30 the DMA platform for each sequential test and to ensure the orientation of the tubules
31 remained constant in relation to the probe.
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39 This study found no significant change in $\tan \delta$ and SM values with time (over 40 minutes)
40 for dentin bars immersed in saline at 26°C, as anticipated from thermo-mechanical studies
41 (12). Those immersed in saline at 60°C also showed no significant change in either $\tan \delta$ or
42 SM over time, however, the saline 80°C group, although showing no significant change in
43 SM, indicated a significant albeit small increase in $\tan \delta$ with time. The minor change in
44 viscous behaviour was clinically insignificant in respect of the absolute $\tan \delta$ values.
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54 Temperature-mediated collagen denaturation is dependent on hydration, neutral salt
55 concentration and confinement of the collagen molecules (14, 23). Unconfined collagen may
56 begin denaturing at around 60°C (23) but is substantially affected by hydration (15). In the
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1 present study, dehydration was not a risk because of constant water saturation but the
2 molecules are confined within mineralised structures, which is said to confer a stabilising
3 effect on thermal denaturation by a so-called “polymer-in-a-box” mechanism (15).
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7 Static mechanical properties may undergo a linear decrease with increasing temperature
8 from 0-80°C (24), although flexural strength and microtensile strength increased with
9 increasing temperature from 50°C to 200°C (25). These changes are reversed upon
10 rehydration and have been attributed to dehydration and the formation of cross-linkages that
11 are reversible upon rehydration (26). Yamashita *et al.* (27) assessed the viscoelasticity of
12 bone heated to 100°C or 200°C with DMA and concluded that heat was not the main
13 contributor to the increased viscoelasticity of bone. The observed increase in viscoelastic
14 behaviour may reflect increasing compliance of the collagen network with increase in
15 temperature and greater absorption of water beginning at 60°C to around 80°C, possibly
16 enhanced by changes in collagen conformation (28, 29).
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31 Dentin bars immersed in NaOCl at 60°C exhibited a significant decrease in SM and an
32 appreciable increase in $\tan \delta$, while those in the NaOCl 80°C showed significant decreases
33 in SM coupled with significant increases in $\tan \delta$. Notwithstanding, the individual specimen
34 variations, the change in the viscoelastic behaviour of dentin in these two groups can clearly
35 be attributed to the heated NaOCl. The detectable changes in the viscoelastic properties of
36 dentin in the heated NaOCl groups may be explained by NaOCl-mediated dentin surface
37 depletion of collagen and deeper denaturation of collagen within dentin (8). These would be
38 manifested as alterations in chemistry and structure of collagen (8, 12).
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50 Collagen denaturation by heat is mediated by unfolding of its triple helices into individual
51 helices due to disruption of hydrogen bonds between the polypeptide chains; the individual
52 helices may then unfold into random coils due to disruption of intra-helical hydrogen bonds
53 (12). Such unfolding may enhance water flow and hydration of the exposed collagen matrix,
54 increasing viscoelastic behaviour (28).
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1 The decrease in SM also signals some effect on the mineral matrix or mineral-collagen
2 interaction (30). Whilst it is known that mineral may be lost from dentine under saline (31) or
3 NaOCl (32) immersion, this is unlikely to be the mechanism at play. It is more likely that the
4 heated NaOCl has a direct effect on the interface between mineral and collagen. This is
5 consistent with the effect of saline at 80°C, where there may be a mild effect on the
6 collagen-mineral bonding interface through increase in the mean length of vibrating chain
7 segments (29). Collagen shrinks on heating at 70°C in saline solution but falls apart at
8 temperatures higher than 80°C (16). In the presence of NaOCl, the effect is likely enhanced
9 by deeper penetration of NaOCl into dentin and thus probable damage to protein interface
10 with the mineral structure (12). This may cause anelastic behaviour in the mineral matrix
11 (29), coupled with greater damping effect. The potential clinical relevance of these two
12 phenomenon is that the increased viscosity would cause greater strain, whilst the loss of
13 elasticity or anelastic behaviour may delay recovery. This enhanced hysteresis, may upon
14 sustained loading cause or support microcracking and subsequent structure failure (33).

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32 The above observed effects on viscoelastic properties are likely to be a function of
33 dependent rather than independent effects between the medium and temperature given the
34 negligible and insignificant effects of heated saline and unheated NaOCl on SM or $\tan \delta$.
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40 In the present experimental model, the temperature was effectively maintained at the
41 designated level but when heated NaOCl is delivered into the root canal system, the
42 temperature may drop in the syringe within 4 minutes (17). Although, heat transfer into
43 dentin may be limited (34), temperature changes to 60°C can thermally induce deformation
44 of human dentin (35). Therefore, further investigation of the depth and nature of chemical
45 changes *in situ* within roots, associated with heated NaOCl irrigation is merited, together with
46 their effects on tooth surface strain (6).

57 **Conclusions**

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Immersion of standardized dentin bars in saline solution at 80°C resulted in small but significant increase in their $\tan \delta$ with time. Immersion in NaOCl solutions heated to 60°C or 80°C, significantly reduced storage modulus and increased $\tan \delta$ with time.

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Figure caption

Figure 1: Means (solid lines) and upper and lower limits of 95% confidence intervals (dotted lines) of storage modulus (GPa) or tan delta plotted against time (minutes), by test medium and temperature

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Table captions

Table 1: Test and control group composition.

Table 2: Multivariable GEE model incorporating Storage modulus as the dependent variable, and test medium and duration of immersion as the independent variables.

Table 3: Multivariable GEE model incorporating Tan δ as the dependent variable, and test medium and duration of immersion as the independent variables.

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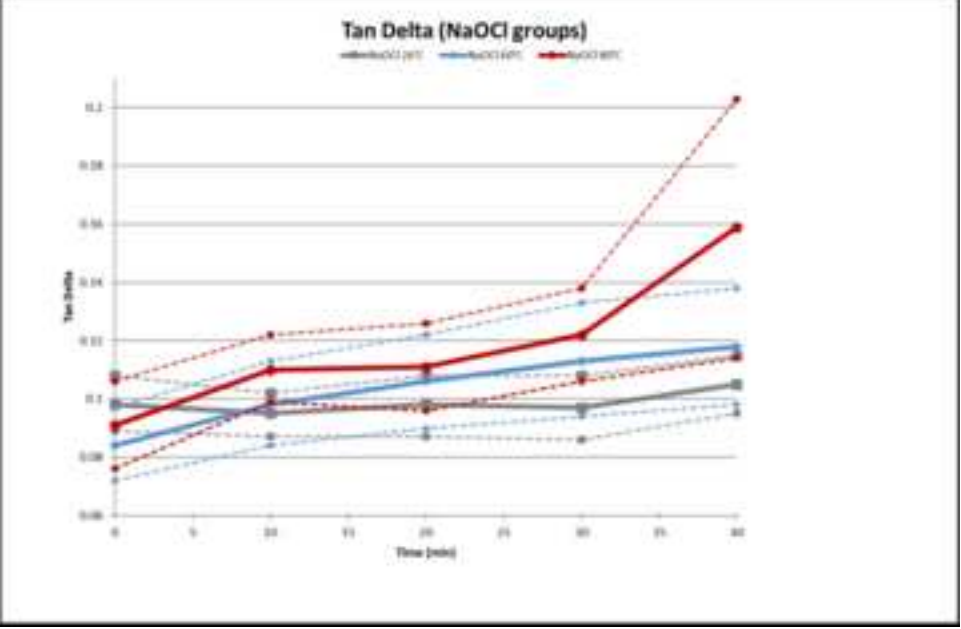
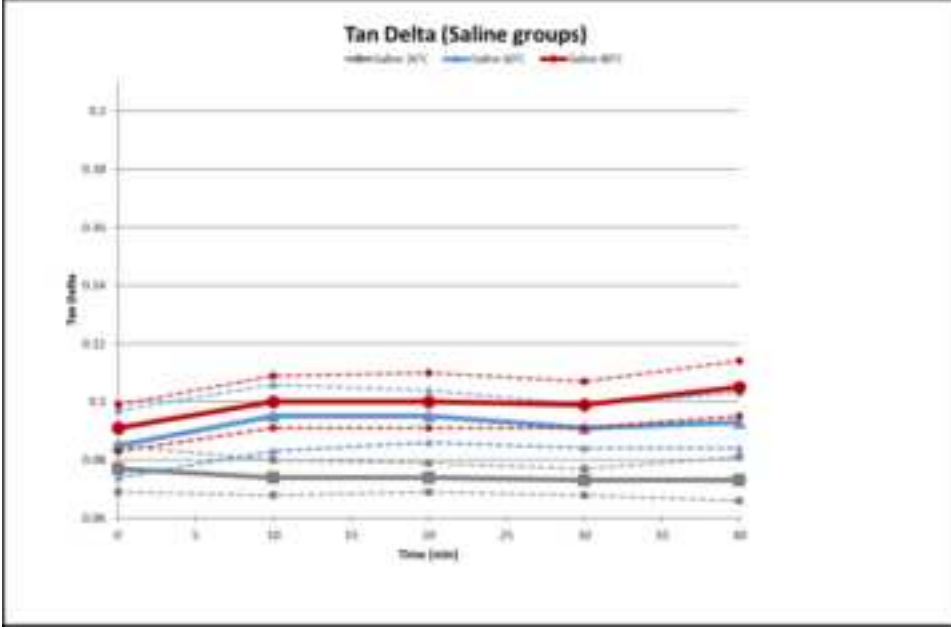
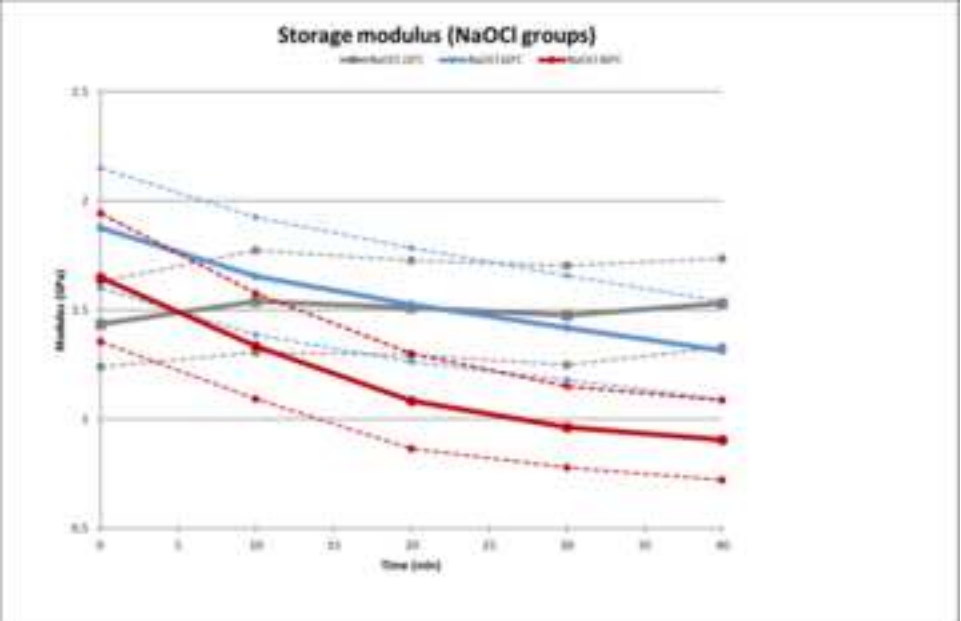
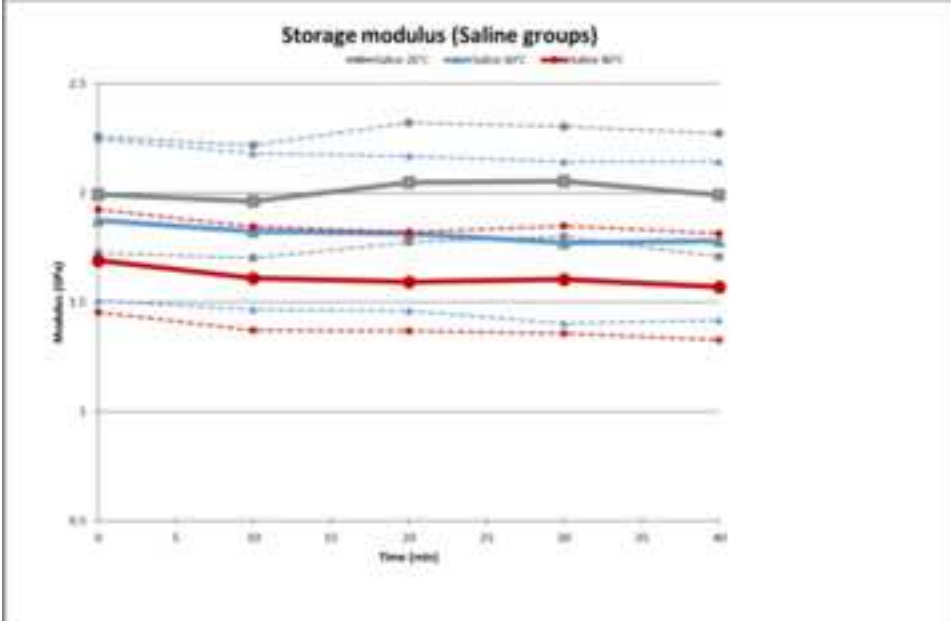


Table 1: Test and control group composition.

Groups	Test medium	Temperature	No. of specimens	Immersion time (mins)	Loading time (min)
1 (control)	Saline (0.9%)	26°C	15	40 (4×10)	1
2	Saline (0.9%)	60°C	15	40 (4×10)	1
3	Saline (0.9%)	80°C	15	40 (4×10)	1
4	NaOCl (5%)	26°C	15	40 (4×10)	1
5	NaOCl (5%)	60°C	15	40 (4×10)	1
6	NaOCl (5%)	80°C	15	40 (4×10)	1

Table 2: Multivariable GEE model incorporating Storage modulus as the dependent variable, and test medium and duration of immersion as the independent variables

Variable	Coefficient	95% Wald Confidence Interval		P value
		Lower	Upper	
Test medium				
Saline 26°C (Ref)	-	-	-	-
Saline 60°C	-0.130	-0.529	0.270	0.5
Saline 80°C	-0.329	-0.643	-0.014	0.04
NaOCl 26°C	-0.518	-0.820	-0.216	0.001
NaOCl 60°C	-0.162	-0.506	0.182	0.4
NaOCl 80°C	-0.432	-0.770	-0.093	0.01
Time (minute)				
Saline 26°C*Time	0.001	-0.002	0.004	0.6
Saline 60°C*Time	-0.003	-0.007	<0.0001	0.09
Saline 80°C*Time	-0.003	-0.007	<0.0001	0.08
NaOCl 26°C*Time	<0.0001	-0.004	0.005	0.8
NaOCl 60°C*Time	-0.014	-0.019	-0.010	<0.0001
NaOCl 80°C*Time	-0.019	-0.025	-0.014	<0.0001

Time = duration of immersion; * = interaction of test solution with duration of exposure

Table 3: Multivariable GEE model incorporating Tan δ as the dependent variable, and test medium and duration of immersion as the independent variables.

Variable	Coefficient	95% Wald Confidence Interval		P value
		Lower	Upper	
Test medium				
Saline 26°C (Ref)	-	-	-	-
Saline 60°C	0.014	0.002	0.025	0.02
Saline 80°C	0.018	0.009	0.026	<0.0001
NaOCl 26°C	0.019	0.011	0.028	<0.0001
NaOCl 60°C	0.011	<0.0001	0.023	0.06
NaOCl 80°C	0.013	0.003	0.024	0.02
Time (minute)				
Saline 26°C*Time	<0.0001	<0.0001	<0.0001	0.3
Saline 60°C*Time	<0.0001	<0.0001	<0.0001	0.4
Saline 80°C*Time	<0.0001	<0.0001	0.001	0.04
NaOCl 26°C*Time	<0.0001	<0.0001	<0.0001	0.3
NaOCl 60°C*Time	0.001	0.001	0.001	0.001
NaOCl 80°C*Time	0.002	0.001	0.002	<0.0001

Time = duration of immersion; * = interaction of test solution with duration of exposure