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Randomized clinical trial to determine if changes in dentine tubule occlusion visualized by SEM of replica impressions correlate with pain scores

Joon Seong BDS, PhD[1], Maria Davies BSc, PhD[1], Emma L Macdonald[1] BSc, MSc, PhD[1], Nicholas CA Claydon[1] BDS, MScD, PhD[1], Nicola X West BDS, PhD[1]

[1]Periodontology, Clinical Trials Unit, Bristol Dental School, Bristol BS1 2LY, UK.

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Corresponding Author

Prof N. X. West

Periodontology

Clinical Trials Unit,

Bristol Dental School,

Lower Maudlin Street,

Bristol, BS1 2LY, UK

Tel: +44 117 3429638

Fax: +44 117 3424000

Email: n.x.west@bristol.ac.uk

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Abstract.

Purpose: To quantify dentine tubule occlusion and correlate this with pain reduction in vivo. **Methods:** This was a single-center, randomized two treatment, examiner-blind, parallel study. 20 participants with confirmed dentine hypersensitivity (DH) were evaluated by Schiff Air Blast, VAS Air Blast and replica impression of the tooth surface to visualize tubule occlusion at baseline and following 4 week twice daily use of either an occluding toothpaste (8% strontium acetate, 1040 ppm fluoride) or a non-occluding toothpaste (1450ppm fluoride). **Results:** Both treatments increased tubule occlusion significantly from baseline to 4 weeks ($p = 0.01$) with significant decreases in pain score only seen with the occluding toothpaste (Schiff, $p = 0.01$; VAS, $p = 0.01$). Schiff pain score after 4 weeks was markedly reduced following treatment with the occluding toothpaste as compared to the non-occluding toothpaste, ($p = 0.05$) with no significant differences between the pastes for occlusion score or patient reported VAS, although the scores favored the occluding toothpaste.

Clinical Significance: Occlusion scores as obtained by replica impression techniques with SEM imaging correlate significantly with DH pain scores confirming proof of concept. With further refinement, this technique could be used to accurately quantify tubule occlusion in vivo and the associated pain reduction achieved by occluding toothpastes.

Introduction

Dentine hypersensitivity (DH) is defined as pain arising from exposed dentine following a non-noxious stimulus which may be thermal, osmotic or tactile which cannot be attributed to any other dental defect or pathology.[1] The condition is relatively common, with 42% of young adults in Europe reporting DH.[2] DH is quick in onset, short-lived, but arresting with regards to pain intensity [3] and has been shown to affect quality of life.[4] In a recent study 28.4% of sufferers stated that their DH pain was either important or very important to them,[2] findings that emphasize the importance of understanding and developing treatments for this condition.

The hydrodynamic theory is the most commonly accepted theory to explain DH and states that triggers of DH cause an outward flow of dentine tubule fluid which triggers a resultant pain response,[5] this suggests DH will only occur if dentine is exposed and patent to the pulp. This theory is supported by the finding that hypersensitive dentine contains more and larger patent dentine tubules than non-sensitive dentine.[6,7] Furthermore, the smear layer has been shown to occlude dentine tubules[8] and it is often absent in hypersensitive dentine.[7] The general acceptance of this theory as the mechanism underlying DH has led to the development of treatments designed to occlude dentine tubules.

Toothpastes are an obvious choice for delivering agents designed to treat DH as they are easy to use at home and can occlude patent tubules. The agents most commonly included to treat the symptoms of DH are, strontium acetate, calcium sodium phosphosilicate (CSPS), stannous fluoride and arginine calcium carbonate.[9] Confirmation that toothpastes containing these agents are able to occlude dentine tubules has been obtained both in vitro and in situ,[10-12] and clinical studies in vivo have demonstrated that they are able to reduce DH pain.[13-16] However, due to the difficulties of detecting tubule occlusion in a clinical environment there are a limited number of studies to date that have examined concurrent tubule occlusion and pain relief in vivo.

The replica technique in which an impression of the tooth surface is made which can subsequently be viewed under a scanning electron microscope (SEM), first used to successfully visualize dentine tubules in vitro,[17] can be used to investigate tubule occlusion in vivo, although few studies have used this methodology. It has been employed to investigate the tubule occlusion efficacies of professionally applied products and it was demonstrated that bonded resins that reduced DH pain also occluded dentine tubules.[18] However, baseline sensitivity elicited by both stimuli was only mild in the majority of

study teeth and a significant reduction in pain was reported for the placebo immediately after treatment indicating the improvements observed could be simply due to the placebo effect. However, differences in pain response between placebo and test products were obtained after one month but no clinician assessed DH response was included in the study and tubule occlusion was not quantified, so a correlation between this and pain score was not possible.[18] A study that examined the efficacy of professionally applied Gluma and a biomimetic agent for the treatment of DH using the replica technique similarly relied exclusively on patient reported pain. [19] It was demonstrated that both DH treatments reduced pain, however due to the mode of application it was not possible to blind patients to the treatment received and there was no negative control, thus it is difficult to discount the placebo effect at least for early study time points. While no difference in DH pain was observed between the agents tested at any time point, the anticipated levels of tubule occlusion were observed for only one agent, suggesting that reduction in hypersensitivity did not fully correlate with tubule occlusion.[19] The failure to find good correlation between tubule occlusion could be in part due to the use of a 2 stage replica technique in which the negative replica initially taken was subsequently converted into a positive replica, as every translation of the tooth surface is likely to introduce inaccuracies. In the only other study to use this technique to assess the efficacy of professionally applied treatments in vivo, tubule occlusion following Nd:YAG laser treatment was observed using a one stage negative replica technique, but no pain measurements were made, thus it is not known to what degree the tubule occlusion observed correlated with a reduction in DH pain.[20]

We also have previously conducted a pilot study using the negative replica technique and SEM imaging to investigate the relative efficacies of a strontium acetate DH and a fluoride control toothpaste to reduce DH and occlude dentine tubule in vivo.[21] Although it was possible to visualise some dentine tubule occlusion we failed to demonstrate significant differences between the non-occluding and occluding toothpaste or significantly correlate tubule occlusion with pain score. Indeed pain responses favoured the non-occluding toothpaste. Review of the SEM images showed residual impression material in the dentine tubules. This suggested that impression material had sheared off from the body of the impression during removal from the participants' mouths and acted to occlude the tubules in both treatment groups indicating and observer effect whereby the act of taking the measurement altered the state of tubule occlusion. Subsequent investigation showed that at time points very close to the manufacturer's recommended set time, a weaker strength of the impression

material than expected was observed. Thus the setting time indicated in the manufacturer's instructions, while appropriate in normal dental treatment situations, appeared to be too short for the replica technique and indicated that the material needed to be handled differently when used for this research purpose. Toothpaste residue was also observed on impressions taken soon after treatment affecting the interpretation of images at early time points In addition, artefacts were observed on the tooth surface such as debris and indentations in the replica material that could have been the result of dentine tubule fluid exuded from the tubule during the impression procedure and these adversely affected occlusion scoring.

The results of the above studies overall indicate that the replica[18, 19] and negative replica [20,21] impression technique may be valuable for confirming the association of tubule occlusion with decreased DH pain, providing definitive evidence in support of the hydrodynamic theory. However, they indicate that study methodologies need to be improved if a significant correlation between the degree of tubule occlusion observed and pain score is to be demonstrated. The primary aim of study was to confirm the relationship between dentine tubule occlusion and the reduction of DH. The dentine tubule occlusion and pain reduction capabilities of an occluding toothpaste (Sensodyne® Rapid Relief; 8% strontium acetate, 1040 ppm fluoride) as compared with a toothpaste with non-occluding properties (Colgate® Cavity Protection; 1450ppm fluoride), were also evaluated.

Materials and Methods:

Trial design and selection of participants.

This was a single center, randomized, two treatment parallel study, with treatment blinded to the pain assessor and impression examiner. NHS Research Ethics Committee (REC) approval was gained for the study and the study was carried out according to Good Clinical Practice guidelines as laid down by the Declaration of Helsinki and its later amendments in a UK dental school. Volunteers aged 18 or over that gave written informed consent were invited to a screening appointment where a medical history and oral soft tissue exam were undertaken, and the fulfilment of inclusion and exclusion criteria determined. Eligible participants were those with good general health, healthy gingivae and a minimum of one sensitive tooth without restoration and with exposed dentine at the cervical margin, demonstrating patent dentine tubules as determined at screening with an impression visualized under

the scanning electron microscope. Participants who were pregnant, had xerostomia, signs of gastroesophageal reflux disease, diabetes or an oral health condition that might interfere with study measures were excluded, as were those who were on medications that might interfere with their pain response.

To confirm sensitivity, all teeth that showed signs of exposed dentine at the cervical margin were subjected to a 1 s air blast from a dental triple syringe at 60 ± 5 psi at 21°C ± 5°C directed 10mm perpendicular away from the exposed dentine of the test tooth. Sensitivity was scored by examiner sensitivity Schiff Score;[22] 0, participant does not respond to stimulus; 1, participant responds to stimulus but does not request discontinuation of stimulus; 2, participant responds to stimulus and requests discontinuation or moves from stimulus; 3, participant responds to stimulus, considers stimulus to be painful and requests discontinuation of stimulus. Up to a maximum of 4 teeth with Schiff score 2 or 3 were then assessed for dentine tubule patency, in the event that more than 4 teeth gave Schiff scores of 2 or 3, those which were most sensitive were selected. To confirm dentine tubule patency of these teeth, silicone impressions of the cervical margin of the tooth with exposed dentine were taken using Aquasil Ultra XLV® (Dentsply Sirona, Weybridge, Surrey, UK) and immediately examined under SEM for tubule patency. Although only one tooth per participant was selected for study treatment, impressions of 4 teeth were taken where possible as dentine is susceptible to damage, and this enabled selection of the tooth with the greatest patency for the study. Impressions (negative replicas of the natural tooth) were scored for tubule patency by reference to existing images demonstrating varying degrees of dentine tubule occlusion and their associated tubule occlusion score. A 5 point scoring system was used in which dentine tubules that were fully occluded scored 1, dentine tubules that were mostly occluded scored 2, dentine tubules that were almost equally occluded/unoccluded scored 3, dentine tubules that were mostly unoccluded scored 4 and dentine tubules that were unoccluded scored 5. Only teeth with Examiner Schiff score of 2 or 3 and occlusion score of 4 or more were acceptable for study inclusion. Only one tooth per participant was selected for the treatment phase, and an area of patent tubules identified for further examination following treatment. A light microscope image of the area identified on the impression was taken using the gingival margin of the impression replica as a reference in conjunction with the surface landmarks on the impression of the natural tooth, to allow future imaging post treatment to be carried out in the same area.

Participants with an eligible tooth and who satisfied all other eligibility requirements were enrolled onto the study and given a standard toothbrush and washout toothpaste (Colgate® Cavity Protection, Colgate, Guildford, Surrey, UK) for use twice daily for a minimum of 24 hours before the treatment visit. Only one tooth per participant was selected for study procedures.

Study treatments

Following the screening visit, eligible participants returned to the study site for the start of the treatment period within 14 days of the screening visit. Participants were asked to refrain from eating and drinking with the exception of water in the hour before their visit, and to brush their teeth with the washout toothpaste between 1 and 3 hours prior to their study appointment. Participants were given a visual analogue score (VAS) training exercise to complete and their general ongoing study eligibility was confirmed by an oral soft tissue exam. At this visit the selected tooth was also re-assessed for sensitivity and tubule occlusion using examiner Schiff and SEM tubule occlusion score, only participants whose selected tooth continued to fill the eligibility criteria of Schiff score $\geq$2 and tubule occlusion score $\geq$4 were advanced to the treatment phase. In addition, prior to impressions being taken to assess tubule occlusion, participants were asked to rate their dentine hypersensitivity using VAS. Participants for whom ongoing eligibility was confirmed were randomized to either Sensodyne® Rapid Relief (occluding; GSK, Brentford, Essex, UK), or Colgate® Cavity Protection (non-occluding), using a pre-determined randomization schedule, participants being randomized by study staff in the order in which they were confirmed eligible to continue in the study. Participants were provided with their study toothpaste, a new toothbrush and instructions on product use at home. The decision to use the same positive control (Sensodyne® Rapid Relief) in this study as the previous study was made as there is good evidence for its tubule occluding ability[11], and its ability to provide relief from DH [14, 23] and it was felt that the poor result obtained as compared to the control toothpaste in the pilot study was soley due to technical issues encountered with the replica impressions. A different standard fluoride control toothpaste was selected at random.

During the treatment phase of the study participants brushed with the study product twice daily for two timed minutes for 4 weeks, after which they returned to the study site for their final visit. The use of other oral health care products during this time was prohibited, with the exception of occasional use of

floss to remove trapped dietary debris. Participants were asked to refrain from eating and drinking with the exception of water in the hour before their final visit, and to brush their teeth with their study toothpaste between 1 and 3 hours prior to their study appointment. Participants were given VAS refresher training, their selected tooth was assessed for sensitivity by examiner Schiff and VAS, and a final silicone impression was taken to determine dentine tubule occlusion.

Taking impressions

Throughout the study replica impressions were taken immediately after sensitivity had been scored. Prior to taking an impression the surface of the selected tooth was wiped gently to remove any oral debris and dried in the air without the use of an air blast, prior to the silicone application, the silicone impression material dispensed from a cartridge was applied directly to the sensitive area of the tooth and allowed to set for 6 minutes (2 minutes longer than the manufacturers guidance for normal clinical use). When set, the silicone was carefully peeled from the tooth, with no pressure applied during the set of the material. One replica impression per selected tooth was taken at each study time point. Prior to SEM analysis, replica impressions were disinfected in 1000 ppm available chlorine solution for 10 min, then rinsed well under running water. The replica impression of the sensitive area was imaged directly using SEM at 2000X magnification using a Phenom bench top SEM (Phenom-World BV, Eindhoven, The Netherlands) without the need to prepare a positive replica which by virtue of adding a second impression step might introduce additional error. The SEM imaging and classification was carried out by a single appropriately trained examiner blind to the treatment that had been applied to the tooth from which the replica impression had been obtained. The examiner was trained to recognize dentine tubule occlusion as visualized with the replica impression technique across the range of scores (1-5), with results validated against standardized image scores, a weighted Kappa coefficient (κ) using the Fleiss-Cohen method of weighting to assess examiner reliability. The reliability was deemed excellent ($\kappa > 0.75$).

Statistical methods

This was a preliminary study to determine if replica impressions visualized by SEM could detect changes in tubule patency in sensitive teeth treated with an occluding or non-occluding toothpaste. The sample size was not based upon statistically powered sample size calculations to detect clinically

relevant differences between treatment groups, but was considered adequate to provide useful information for the design of future studies. It was anticipated that if 20 participants were randomized this should ensure that at least 15 participants completed the study.

Outcome measures were dentine tubule occlusion, evaporative air Schiff Scores and patient reported VAS score after 4 weeks of treatment, and change from baseline at 4 weeks from treatment.

Dentine tubule occlusion and evaporative air Schiff Scores were analyzed using non-parametric methods. A Wilcoxon rank sum test was performed to test for differences between treatments and Wilcoxon signed rank test was performed to look at changes from baseline visit within treatment. The relationship between Schiff score, VAS and the dentine tubule occlusion score was assessed using the Pearson's correlation coefficient. The statistical analysis was carried out using the SPSS version 21.

Results

A total of 20 participants were eligible for the study and were randomized, 10 receiving each treatment, all participants randomized to study products completed the study between August and December 2014. There were 3 male and 17 female participants, with a mean age of 42.1 years. No protocol deviations or adverse events were recorded during the study thus the intention to treat and per protocol populations were the same.

To determine whether tubule occlusion correlated with dentine hypersensitivity pain scores, all the baseline and 4 week data from each treatment was considered together. Evaporative air Schiff pain scores correlated significantly with patient reported VAS scores, $p = 0.015$. Occlusion scores also correlated significantly with both pain scores, $p = 0.031$ and $p = 0.045$ for examiner reported Schiff and patient reported VAS respectively.

Analysis within treatments demonstrated that for both treatments tubule occlusion increased significantly from baseline to 4 weeks (SRR, $p = 0.01$; CCP, $p = 0.01$; table 1). However, significant decreases in pain score were only seen with the occluding toothpaste SRR (Schiff, $p = 0.01$; VAS, $p = 0.01$).

Comparing treatments at each time point, it was confirmed that there were no significant differences between the groups randomized to SRR or CCP prior to treatment for any measure tested. After 4

weeks treatment pain score as assessed by examiner Schiff was significantly reduced following treatment with SRR as compared to CCP, ($p = 0.05$; table 2). There were no significant differences between toothpastes for occlusion score or patient reported VAS although the scores favored the occluding toothpaste.

Discussion

This study aimed to further develop the replica impression technique and confirm that tubule occlusion captured by the technique in vivo correlated with DH pain. Further the study sought to determine whether the technique was able to differentiate between the degrees of tubule occlusion achieved following treatment with an occluding or non-occluding toothpaste.

The methodology of the present study was based on that of our pilot study[21] but modified to address the technical issues encountered as follows: prior to impression taking, following a gentle wipe the tooth was dried by isolating and leaving to dry naturally in the environment, as opposed to being dried with an air blast, to prevent increased outward dentine tubule fluid flow; the impression material was allowed to set for 6 min as compared 4min and handled with extreme care to avoid material shearing off within tubules; and no impressions were taken immediately after use of toothpaste to avoid issues associated with toothpaste debris. The control toothpaste was also changed in ensure the abrasive was not contributing to the reduction in DH by tubule occlusion.

The study successfully demonstrated that dentine tubule occlusion scores obtained from replica impressions taken using Aquasil Ultra XLV impression material significantly correlated with DH pain scores in vivo. This finding provides direct evidence to support the hydrodynamic theory of Brannstrom.[5]

The findings are broadly in agreement with one replica study that demonstrated that professionally applied dentine desensitizing agents reduced DH pain and occluded dentine tubules,[18] However, this study did not quantify the tubule occlusion observed, so a quantitative correlation with pain score to provide direct evidence for the hydrodynamic theory was not possible. The findings are also supported by a study in which tubule occlusion by the smear layer was associated with non-sensitive dentine,[7] but the degree of DH pain was not scored, so whether occlusion correlated significantly with

DH pain could not be determined. By contrast Guentsch et al[19] demonstrated that while both professional treatments tested caused a similar reduction in pain scores, for one of the treatments tubule occlusion was only partial.[1] Similarly, in a previous study, a significant correlation between tubule occlusion and pain was not observed, although the correlation was positive. [21]

The present study demonstrated that DH pain scores in the SRR (occluding) group decreased significantly by 4 weeks. By contrast, and as predicted, the control (non-occluding) toothpaste did not reduce pain scores significantly over this period. The demonstration that SRR (occluding) reduced DH pain is in agreement with previous clinical studies which have demonstrated strontium acetate containing toothpastes reduce pain at time points from 3 days to 8 weeks.[14, 23]

By contrast to the findings for pain reduction, in the present study it was demonstrated that both toothpastes resulted in significant tubule occlusion after 4 weeks, the results slightly favoring the occluding toothpaste. The data for SRR, the occluding toothpaste is as expected, as it has been demonstrated to occlude dentine tubules in vitro and in situ.[24] But in contrast to the current study, previous studies have demonstrated that strontium acetate occluding toothpastes are able to occlude dentine tubules better than non-occluding control toothpastes.[12, 25] It is possible that in the present study impression material was retained became lodged in open dentine tubules, and snapped off when the impression was removed from the participant's mouth resulting in 'false occlusion'. If this was the case it would be more likely to occur in participants in the non-occluding group who would be anticipated to have more patent dentine tubules. However, in contrast to our previous study[21] obvious signs that impression material had lodged in dentine tubules were missing from the SEM images, likely a result of the additional setting time and careful handling of the material, however it is possible that the impression material snapped off a few microns into the tubule orifice.

Other artefacts that interfered with scoring were also present on some replica impressions. While the tooth surface was carefully wiped prior to the impression to remove debris, inevitably a small amount of debris was observed. In addition, patterns in the replica impression that could have been caused by outward dentine fluid movement from unoccluded tubules were seen on some impressions. On a replica impression, outward fluid movement from an unoccluded dentine tubule leaves an indentation, as opposed to a finger of impression material that is seen when the material penetrates unoccluded tubules, thus this also can result in 'false positives'. This artefact, therefore could also be responsible

for the higher than anticipated occlusion scores obtained in the control toothpaste, which consequently did not fully reflect the pain scores. These scores obtained with this toothpaste not being significantly reduced in the control group after 4 weeks for either assessment measure.

Another possibility to reconcile the occlusion and pain scores for the control paste is whether there was a placebo effect with respect to the pain score, as substantial placebo effects have been previously reported for dentine hypersensitivity studies[30]. However, the duration of the present study was relatively long, and both toothpastes were used twice a day in line with normal oral healthcare regimes so the likelihood of participants behaving differently because they were on a clinical trial was reduced. Further both patient reported VAS and examiner assessed Schiff scores favoured the occluding toothpaste, with the improvements in VAS for the occluding toothpaste being much greater than those observed for the control toothpaste, even though the difference was only significant for Schiff. It is recognised that pain measurements are subjective[31] with pain being hard to quantify[32]. However, the dental examiner was experienced in judging patient response to pain, when recording Schiff score, and to what degree participants are able to modify their response to a trigger of DH is not clear. While VAS scores are more readily by the placebo effect, it is important to record patient reported pain. VAS has been shown to be work well oral surgery post operative pain,[33] and recently in a DH study has been shown to distinguish between cold and warm water stimuli similar to a labelled magnitude scale and was preferred by some of the study participants,[34] supporting its use in DH studies.

In the current study there were no significant differences between the toothpastes after 4 weeks of treatment for occlusion score or VAS, however the data favored the occluding toothpaste, and there was a significant difference between toothpastes as scored by Schiff ($p=0.05$). Other clinical studies have demonstrated that strontium acetate containing toothpastes provide significantly better pain relief than fluoride control toothpastes at least in the short term.[26] As this was an exploratory study it was not powered to detect differences between treatments, but to inform future studies. We were, however, able to demonstrate significant improvements in pain score for SRR but not CCP. Perhaps with more participants, differences between the two products after treatment would become more apparent, although two other studies have also demonstrated no differences in the pain relief achieved following treatment of DH with SRR as compared to a fluoride control after 7 days and 2, 4, and 8 weeks.[27, 28]

Taken together the results of this study confirm that occlusion scores as obtained by replica impression techniques with SEM imaging correlate significantly with DH pain score, but the technique still needs refining to detect significant differences in tubule occlusion between toothpastes in vivo. The technique and impression material used in the present study has been successfully used to follow the course of early enamel erosion and recovery,[29] but improvements in impression materials shear properties are required before replication of dentine tubule status can be achieved to an accuracy that allows for clear distinction between occluding and non-occluding toothpastes.

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Table 1: Within treatment analysis of occlusion and sensitivity scores from baseline to 4 weeks

		Baseline Visit		4 Weeks (Final Visit)		p value
		Mean	Standard Deviation	Mean	Standard Deviation	
Sensodyne ® Rapid Relief	Occlusion	4.4	0.52	2.5	0.97	0.01
	Schiff	2.0	1.15	0.5	0.71	0.05
	VAS	66.9	19.05	40.4	25.44	0.01
Colgate® Cavity Protection	Occlusion	4	0.00	2.7	0.95	0.01
	Schiff	1.6	0.84	1.5	1.08	0.71
	VAS	47.1	24.41	49.7	28.98	0.92

Table 2. Differences in occlusion, examiner Schiff and self-reported VAS score between SRR and CCP at all study time points

		Sensodyne® Rapid Relief			Colgate® Cavity Protection			Sensodyne vs Colgate (p value)
		Number	Mean	Standard Deviation	Number	Mean	Standard Deviation	
Screening	Occlusion	10	4.8	0.42	10	4.5	0.53	0.28
	Schiff	10	2.1	0.57	10	2	0.00	0.74
Baseline Visit	Occlusion	10	4.4	0.52	10	4.0	0.00	0.14
	Schiff	10	2.0	1.15	10	1.6	0.84	0.25
	VAS	10	66.9	19.05	10	47.1	24.41	0.11
4 Weeks (Final Visit)	Occlusion	10	2.5	0.97	10	2.7	0.95	0.63
	Schiff	10	0.5	0.71	10	1.5	1.08	0.05
	VAS	10	40.4	25.44	10	49.7	28.98	0.48