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**The Ability of a Potassium Oxalate Gel Strip to Occlude Human Dentine Tubules; A Novel in vitro: in situ Study**

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**Short Title.**

Oxalate strip occlusion of dentine fluid flow in situ

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Dentine tubules, Potassium oxalate, dentine hypersensitivity, tubule occlusion, flow cell, Pashley model

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**Abstract**

Objectives: To determine if an oxalate strip reduced fluid flow in dentine samples and whether this reduction was maintained following a 14 day intra-oral period.

Methods: Dentine tubule fluid flow was measured by a modified Pashley cell in 40 acid-etched dentine discs 1mm thick, diameter >10mm, with an acquired pellicle, pre-equilibrated with Hartmann's solution and conditioned by toothbrushing, pre and post treatment (10min) with an oxalate (3.14%) gel strip or no treatment. One control and one test sample were exposed in-situ for 14 days to the oral environment in 20 healthy adult volunteers, and fluid flow re-measured. The appliance containing the two samples was removed for brushing with water after mealtimes when the participant brushed their teeth and for a 2min daily soak in chlorhexidine.

Results: Fluid flow rate was reduced significantly immediately following treatment with the oxalate strip compared to baseline flow rate by 58%. Following 14 days in-situ oral environment phase, a significant further reduction in fluid flow compared to baseline was identified in both control and oxalate strip treated samples, both ($p<0.0001$), but the reduction was greater in the test samples, 94% vs 87%, $p<0.01$.

Conclusions: This novel investigation is the first to show fluid flow measurement using the Pashley model in dentine samples that have been housed in the mouth for 14 days. Treatment with an oxalate strip designed for dentine hypersensitivity alleviation reduced dentine fluid flow more than control providing evidence that the oxalate treatment withstood the oral environment over a prolonged time.

Clinical Significance

This study demonstrated the efficacy and durability of the oxalate precipitate over a 14 day period in achieving and maintaining dentine tubule occlusion when participants had no dietary restrictions. This demonstrates the suitability of the oxalate strip for the treatment of patients suffering from dentine hypersensitivity pain.

## 1. Introduction

Human dentine is a vital tooth tissue, consisting of numerous, densely packed dentinal tubules occupied by odontoblastic processes, collagen fibres and proteins with dentinal fluid flowing through the structure from the pulp to the oral environment. The diameter and density of the tubules change along the thickness of dentine. As the distance from the pulp increases, the diameter of the tubules decreases being in the order of 1-2 µm at the dentine outer edge, with lateral branching particularly at their terminal ends [1]. The dentine of a healthy tooth is protected by enamel on the crown and cementum on the root. These mineral coatings offer protection which can be physically and/or chemically removed over time. The most common aetiological factors that have the potential to remove the protective surfaces are oral impacts such as dietary acids causing erosive tooth wear and overzealous toothbrushing resulting in abrasive lesions. It was shown that surface roughness in dentine samples exposed to an erosive challenge increased significantly and that following toothbrushing with toothpaste, a force of 400g increased roughness significantly more than a force of 100g [2]. Similarly, a negative impact of high brushing force on tubule patency has been demonstrated, the number of patent dentine tubules significantly increasing following brushing at 400g with or without acid challenge, whereas brushing with a force of 100g reduced the mean number of patent tubules [3]. Thus, both individually and in combination erosive and abrasive challenges are able to result in dentinal tubule exposure to the oral environment [4].

Dentine tubules are filled with fluid which affords a degree of permeability to the tooth structure, possibly as a shock absorber. However when exposed to the oral environment a number of stimuli are able to induce an increase in dentine tubule fluid flow rate which in turn is thought to be responsible for nociceptor activation in the dental pulp [5] and subsequent neuron activation reported by individuals as tooth pain[6]. This hydrodynamic theory provides a mechanism for the clinically diagnosed condition of dentine hypersensitivity (DH). DH is a common oral pain condition affecting quality of life with a prevalence of 42% in young adults in Europe [7]. It is defined as "pain arising from exposed dentine in response to stimuli, typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other form of dental defect or pathology" [8].

Clinical DH studies have demonstrated teeth eliciting pain have dentine tubules patent from the oral environment to the pulp [9] and show as many as eight times more patent tubules than non-sensitive teeth with the former tubules also being twice as wide [10]. Further, hydraulic conductance studies have highlighted the importance of dentine tubule patency in facilitating fluid movement [11]. Once the dentine is exposed it is not possible to recover the lost enamel or cementum, however the dentine tubules can be occluded with toothpaste ingredients [12] or dental restorative materials [13] reducing bulk flow rate by tubule narrowing or orifice coverage, this being the current preferred treatment modality for DH.

Methods to examine dentine tubule patency commonly involve imaging the dentine using techniques such as scanning electron microscopy (SEM), Confocal Laser Scanning Microscopy (CLSM), tandem scanning microscopy or Atomic Force Microscopy (AFM) [14,15]. Initial studies used SEM, images being scored on an ordinal scale by examiners blinded to treatment [16]. The development of an innovative software capable of determining tubule patency from SEM and tandem microscopy images introduced a more objective scoring method [14]. In a recent study CLSM was used with a validated computer algorithm to score surface tubule occlusion, while SEM was used together with Energy Dispersive X-Ray Spectroscopy (EDX) to visualise occluding deposits deeper within the dentine tubules and AFM was used to evaluate both tubule patency and inter-tubular roughness [15]. This study also examined contact profilometry as a method of measuring tubule occlusion and demonstrated a negative correlation between the surface roughness detected and tubule patency.

The Pashley model is a well-recognised method to determine dentine permeability in an in vitro setting [17]. Typically dentine discs are prepared with the surface perpendicular to the tubules which are exposed to an acid etch solution to reveal patent tubules. Pressurised fluid flow through dentinal tubules is then quantified and can be used to determine fluid flow before and after surface treatment in vitro. This model can therefore be used to determine the efficacy of occluding agents to potentially relieve the pain of DH whilst bearing in mind that it is based on in vitro measurements that should only be extrapolated to the clinical situation with caution. To the authors knowledge to date no studies have investigated the change in fluid flow of dentine discs that have also been housed in the oral environment. One study [18] has attempted to modify the model by preparing samples that are more closely related to the clinical situation by preparing a cavity in the crevicular area of a whole tooth but this model was not exposed to the oral environment.

Over the counter DH treatment is the most efficient and immediately effective way to deliver tubule occluding agents to the tooth surface as they are applied twice daily as part of a good oral hygiene routine. There are a number of agents which are formulated into dental products aiming to reduce the sensation of DH by dentine tubule occlusion. These agents include stannous ions [19], arginine and calcium carbonate [20], strontium ions [21], calcium sodium phosphosilicate [22] and oxalate salts [23,24]. Oxalate salts have been found particularly effective in reducing dentinal fluid flow when applied in the form of potassium oxalate delivered as a gel [24-26]. The application of oxalate-based gel leads to the formation of calcium oxalate crystalline precipitates that can form on the surface and within dentine tubules. Oxalate reacts with calcium that is freely available at the mineral surface and within salivary and dentinal fluid to form calcium oxalate precipitates [27,28].

The aims of this study were to use a modified Pashley cell to determine (1) the change in fluid flow through dentine treated with a potassium oxalate gel strip directly applied onto the dentine disc surface in vitro and (2) the durability of the potassium oxalate in reducing dentine permeability after human participants have worn appliances housing the oxalate pre-treated dentine discs in situ for 14 days. A further aim was to determine whether any tubule occlusion observed was due to surface or subsurface oxalate deposits using SEM with EDX.

## 2. Materials and Methods

### *2.1. Study Design*

This study was a single centre, single blind (with respect to the participant who wore the appliance), negative control, split mouth design study. Ethical approval was given by the South West - Exeter Research Ethics Committee, under the REC REF: 16 SW 0050. A total of 20 female and male participants at least 18 years of age who were in good health were recruited from a UK Dental School, Hospital Trust and the local area. Informed consent was taken from all participants prior to the start of the study. Exclusion criteria included periodontal disease, a history of kidney stones, pregnancy, fixed orthodontic appliance, any disease or conditions that might interfere with the subject safely completing the study, inability to undergo study procedures or a history of allergies or hypersensitivity to ingredients within the commercial products used.

A baseline in vitro phase in which samples (dentine discs) were treated with or without potassium oxalate gel preceded an in situ phase where one treated and one untreated disc chosen at random were placed into an oral appliance prior to placement in a participants mouth for a 14 day (±3 days) in situ study.

### *2.2. Dentine Disc Preparation*

Dentine discs with a thickness of 1 mm (+/- 0.1 mm), a diameter >10 mm and sectioned parallel to the occlusal surface were prepared from human third molars obtained from an NHS Research Ethics

Committee approved tooth tissue bank (REC REF: 11/NI/0145, HTA licence 12200). A Micro Slice Annular Saw (Ultra Tec Manufacturing Inc, Santa Ana, USA) with direct water irrigation was used to section the teeth. Care was taken to avoid the presence of enamel on the side closest to the occlusal plane or the pulpal horns on the side closest to the cervical margin when sectioning the dentine discs. The cervical side of the disc was identified with a tape marker on the enamel surrounding the dentine to allow orientation of the specimen. Both sides of the discs were polished by hand using silicon carbide paper (Kemet International Ltd, Maidstone, UK) up to p2500 grade using deionised water as lubricant. The dentine discs were etched in citric acid (6%) under ultrasonication (Minerva, Ultrawave Ltd, Cardiff, UK) for 2 minutes each side before being rinsed with Hartmann's solution [sodium chloride (6 g, 102.6 mmol), sodium lactate (3.12 g, 27.8 mmol), potassium chloride (0.4 g, 5.3 mmol) calcium chloride (0.27 g, 2.4 mmol) in water (1000ml)].

### *2.3. Pellicle Development*

Prior to in vitro baseline fluid flow readings being taken using the Pashley cell, an acquired pellicle was developed on each dentine disc using saliva collected from participants that would wear the appliance housing that disc during the in situ phase of the study. All participants had provided informed consent prior to donating saliva. Participants were asked to provide 5 ml of unstimulated saliva by expectorating into a universal container. 0.5 ml of whole human saliva was placed on top of the dentine disc (treatment side) and left for 1 hour prior to being rinsed using Hartmann's solution (Baxter Healthcare Ltd, Norfolk, UK).

### *2.4. Pashley Cell Apparatus*

A custom built modified Pashley cell was used to determine the fluid flow through the dentine discs before treatment and following in vitro treatment with a potassium oxalate (3.14 %) strip (Crest® Sensi Stop Strip, Procter and Gamble Inc, Ohio, USA). The difference in flow rate between discs that had been treated and those which had not been treated with the potassium oxalate strips was also determined following 14 days incubation in the oral environment. The dentine disc was placed in a sealed cell so that the only path for pressurised liquid was through the dentine tubules. Sterilised Hartmann's solution was used as the flow solution and the flow was set at either 0.4 psi equating to in-vivo pulpal pressure or 30 psi equating to the approximate pressure reported to cause moderate pain in-vivo (Figure 1) [11,29].

The disc was initially equilibrated with Hartmann's solution for 5 minutes set at a pressure of 30 psi. An acquired pellicle was then developed on the dentine surface under a pressure of 0.4 psi prior to a further 5 minutes of equilibration at 30 psi. Following the development of the pellicle layer and equilibration with the Hartmann's solution, the dentine surface was brushed using both a manual and electric toothbrush for a total of 8 minutes to remove any crystallites and condition the surface. Following another 5 minutes of equilibration at 30 psi the dentine surface was brushed again using a manual toothbrush for 30 seconds. Following the conditioning stages the pressure was reduced to 0.4 psi and four baseline flow rate readings were taken by releasing an air bubble into the tubing of the apparatus. The movement of the air bubble represented the flow of solution through the dentine tubules. The dentine surface was brushed for 30 seconds and equilibrated for 2 minutes under a pressure of 30 psi in between each baseline reading. To ensure that brushing force was standardised the individual brushing the sample was trained to brush a test sample on a balance and maintain a force of 120g throughout the brushing time. Calibration was performed every day prior to brushing study samples.

The linear movement of the bubble (*l*) within the tubing over time (*t*) allowed the volumetric flow rate (*Q*) through the disc to be calculated using equation 1.

Equation 1

$$Q = \frac{\pi r_i^2 l}{t}$$

(Where $r_i$ is the inner radius of the tube)

The baseline flow rate was measured as the initial flow rate following etching. Further measurements were expressed as a % reduction from the baseline flow rate as per the following equation 2:

Equation 2

$$\%\ \text{Reduction} = 100\,\frac{(Qp - Qb)}{Qb}$$

(Where *Qp* = mean post-treatment flow and *Qb* = mean baseline flow)

*2.5. Treatment*

Following baseline readings, twenty discs (one per participant) were treated with Crest ® Sensi Stop Strip gel containing potassium oxalate (3.14%) whilst the other twenty discs were left untreated. The gel strip was placed on top of the dentine disc. To achieve accurate coverage of the sample a 9.5mm disc was cut from the strip using a gasket punch and the protective film removed with tweezers to expose the gel, the gel was then applied to the occlusal side of the dentine surface via the opening in the upper cell bracket. Capillary action and small rotation ensured an even spread of gel across the dentine surface, and the disc was left for 10 minutes as per the manufacturer's instructions under a flow pressure of 0.4 psi. Any residual gel was rinsed off using deionised water. After treatment the discs were flushed with Hartmann's solution for 2 minutes at 30 psi before being remeasured for their post treatment flow rate at a flow pressure of 0.4 psi. Four post treatment readings were taken for each disc.

### *2.6. In Situ*

Palatal appliances to house the dentine discs were constructed from moulds taken of each participants' upper teeth and palate. Figure 2 shows an example of the palatal appliance housing the two dentine discs.

The participants were requested to wear the inter-oral appliances housing the two dentine discs (one treated and one untreated) for a period of 14 days including during mealtimes. They were instructed not to consume sticky foodstuff such as chewing gum and toffee for the duration of the study.

Participants were instructed to remove their appliances to brush their teeth twice a day, morning and night using the supplied toothpaste and toothbrush [Crest Decay Protection Toothpaste (1450ppm Fluoride as sodium fluoride)] and Oral B Indicator Toothbrush, The Procter and Gamble Co., Cincinnati, USA). The participants were also asked to rinse and brush their appliance in running water after each mealtime. Site study staff also checked and cleaned the appliance by brushing with water daily. The appliance was also soaked in Corsodyl Daily Mouthwash (GSK, Brentford, UK) for 2 minutes and rinsed with DI water each day before being returned to the participant.

After 14 days the discs were removed and measured using the modified Pashley cell to ascertain the flow rate following 14 days oral wear. Four post wear measurements were taken per disc.

### *2.7. Surface Imaging*

Once all the flow rate measurements had been collected, the dentine discs were fractured for imaging using a desktop Scanning Electron Microscope (Phenom G2 Pro desktop SEM, Phenom World BV, Eindhoven, The Netherlands). The cross section of the disc was imaged to show the penetration of any occluding agent in the tubules.

### *2.8. Statistical analysis*

A Wilcoxon Signed Rank Test and analysis of covariance for split mouth, placebo-controlled design was used to analyse the flow rate measurements. Random effects were included in the covariance structure of the model to account for the within subject variability. Statistical comparisons utilised two-sided testing with a 5% significance level.

## 3. Results

The percentage reduction in flow rate from baseline following treatment with 3.14% potassium oxalate strip is presented in Figure 3. The flow rate as measured using the modified Pashley model following treatment with the potassium oxalate containing strip was reduced by 58 % which was a highly significant reduction from baseline ($p < 0.0001$).

20 participants were enrolled in the in situ study. Their ages ranged from 21 to 61 with an average age of 38 years. 17 of the participants were female and 3 males. There were no adverse events reported for this study and all treatment products were well tolerated. All 20 participants completed the study but one treated disc broke on removal from the appliance. Results from three other treated as well as three non-treated discs were removed from analysis due to the inability to gain a sufficient seal with the disc in the modified Pashley model. 16 treated and 17 non treated discs were analysed.

Post 14 days oral wear the flow rate of the dentine discs treated with the potassium oxalate strip reduced further to 94.1% of the baseline rate as shown in Figure 4. The discs that were left untreated showed a reduction in flow rate of 87.2 % following oral wear which was also highly significant compared to the baseline flow rate ($p < 0.0001$). Following oral wear, the reduction in flow rate was significantly greater for the group that had been treated with the potassium oxalate containing strip ($p = 0.0038$) compared to the untreated discs.

SEM analysis of treated discs that had been housed in the oral appliances for 14 days showed the presence of occluding particles within the tubules (Figure 5 A&B). Occluding particles can be seen up to approximately 30 μm below the surface. The non-treated discs showed no occluding particles within the tubules (Figure 5 C&D).

## 4. Discussion

The results from our novel Pashley cell model study showed that 3.14% potassium oxalate as delivered from a gel strip reduced dentine permeability by 58% which was highly significant compared to the baseline flow rate, and this flow rate was further reduced and sustained in a challenging oral environment.

The Pashley cell model has previously been used to determine the effectiveness of potassium oxalate in reducing fluid flow through dentine discs in vitro with good success [24-26, 28]. However, the novel approach described in the present study demonstrates not only effectiveness of this agent at reduction of fluid flow but also sustainability of the treatment effect when exposed to the acidic and frictional challenges of the oral cavity which occur in daily life. Another in vitro study [28] has shown a reduction in dentine permeability following treatment with a potassium oxalate containing hydrogel of 70% following a 10 minute exposure, but the concentration of the potassium oxalate used was 20% (wt/wt) which was far greater than that used in our study at 3.14% (wt/wt), and is not commercially available. The latter study further investigated the in vitro reduction in permeability of commercially available dentine hypersensitivity products having active agents other than oxalate. Our results compared favourably with the other commercial products which reduced dentine permeability from between 20% and 45% [28].

The initial result from the present study confirmed that the modified Pashley cell was able to effectively determine a reduction in fluid flow following treatment with the potassium oxalate gel strip, however most importantly, this reduction in fluid flow was further increased after the discs had been housed in the oral environment for 14 days. The reduction in fluid flow increased to 94.1% which could be attributed to further formation of calcium oxalate precipitation within and on the surface around the orifice of the patent tubules upon exposure to saliva in situ. The reduction in fluid flow has previously been attributed to the formation of calcium oxalate precipitates within tubules due to fluid flow through the dentine structure [30]. For calcium oxalate to form, potassium exchanges with calcium ions found in the surrounding fluids and precipitates are formed on the dentine surface and within the dentinal tubules. The precipitates of calcium oxalate have been found to have an immediate effect on reducing fluid flow whilst also causing further reduction in dentine permeability over time up to 30 minutes post application of potassium oxalate [26]. The authors of this study hypothesise that this may be due to the fluid flow within the system dislodging some of the loosely bound crystals that formed initially and that these travelled through the tubules and formed further blockages. This phenomenon could have taken place in the present study whilst the discs were housed in the oral environment as well as during the post wear measurement.

The conversion of potassium oxalate into calcium oxalate precipitates to reduce dentine permeability is limited in vitro by the source of available calcium. In standard experiments there may be limited available calcium present due to the absence of saliva within the model. The way that the dentine disc is prepared for in vitro studies can also influence the amount of available calcium, which is dependent on the level of hydration at the surface and how the surface is etched [25]. In the present study, the discs were hydrated prior to treatment using saliva from each participant. Saliva was collected to form a pellicle on the dentine disc prior to treatment with the potassium oxalate strip, mimicking exposed vital dentine in the oral environment. Calcium within the pellicle and the etched dentine surface will have provided a reservoir of available calcium which supports the formation of precipitates of calcium oxalates as responsible for the reduction in fluid flow following treatment. Exposure to the oral environment exposed the dentine surface to a further source of calcium from saliva and this may have allowed further formation of calcium oxalate within the tubules, if the potassium oxalate was still present on the dentine surface.

Another reason for the greater reduction in fluid flow following exposure to the oral environment could be that dietary products and oral debris formed a smear layer on the surface of the dentine with resulting surface coverage of the dentine tubules. The direct surface of the dentine disc was not brushed during the 14 days in the mouth so as to avoid damaging the delicate surface. The results demonstrated that the discs that had been left untreated also showed a reduction in fluid flow and therefore it is believed that dietary and oral debris may have had an influence on the reduction in fluid flow in this phase of the study. Others have also found that the amount of fluid flow reduces over time for dentine discs that have not been pre-treated with an occluding product [26,31]. This is most likely due to a combination of organic and inorganic remnants remaining within the tubules after etching being dislodged by the fluid flow and reducing the diameter of the tubules further down the specimen [26] as well as crystallites forming from the minerals within the flow solution.

We used Hartmann's solution as the flow solution in this study which does include minerals that could precipitate within the tubules. The conditioning stage of brushing the dentine surface prior to measuring the fluid flow was included to remove any crystallites that may have formed on the dentine surface but this would not remove any that had formed within the tubules. Likewise, any crystallites or deposits that may have been forced down the tubules whilst the discs were being housed in the oral environment would not have been removed by the conditioning stage carried out prior to measuring post oral wear. Another study also found that in vitro artificial saliva treated specimens with no active ingredients showed a high reduction in permeability of approximately 60% after 10 minutes and 75% after 20 minutes but following acid treatment this reduction was not retained to the same degree as those treated with potassium oxalate [28]. Further evidence for the acid resistance of oxalate crystals was obtained in an in vitro study where samples were treated with a 3% oxalate gel [25]. In the present study, there was no restriction on consumption of dietary acids, thus samples are likely to have been exposed to a considerable acid challenge. The degree of fluid flow reduction post oral wear was significantly greater for the discs treated with the potassium oxalate gel strip compared to those that had been left untreated which suggests that oral debris was not the only influence on the reduction in fluid flow of the treated discs and that oxalate precipitates were resistant to the dietary acids they were exposed to.

SEM images confirmed the formation of precipitates within the dentine tubules of the treated discs. It was difficult to determine the dentine surface coverage and tubular orifice occlusion with SEM due to oral debris on the treated and non-treated discs. After fracturing the discs, potassium oxalate gel strip treated specimens were clearly identified due to precipitation within the dentine tubules which was not visible in the untreated discs. This is in line with previous results investigating the occluding potential of potassium oxalate [25,28,32]. Other in vitro studies with no external oral influences such as oral debris within the model found that imaging the surface did not reveal a complete coating on the surface of the dentine and tubules appeared occluded in the depth of the tubule rather than at the surface [33]. It is therefore important to investigate the tubule occlusion in the depth of the tubule for this agent as opposed to the surface alone in line with the mode of action of a number of other occluding agents, as this will not reveal the potential occlusion benefit for potassium oxalate [34].

One issue encountered in the present study was sample fracture. To fit and make a seal in the Pashley cell and to allow the measurement dentine fluid flow dentine samples needed to be >1cm in diameter and 1.2 +/- 0.2 mm thick. However these dimensions meant that the dentine samples were fragile and could be damaged by overtightening of the Pashley cell retainer, or when inserting or retrieving the samples from the oral appliance. Furthermore, to best mimic real life, samples were worn continuously for 14 days apart from participants twice-daily brushing and cleaning of the appliance, with the only dietary restriction to avoid very sticky or chewy foods, thus samples may have received a substantial acidic challenge during this time. To try to minimise sample fracture, great care was taken when handling samples and oral appliance design was considered carefully to allow interaction with food and drink while protecting the dentine from large food debris, however fracture rates were still higher than ideal although this was not unexpected. The authors feel that conducting a study of this nature with samples of this size and fragility would have at least this number of sample fractures. To address this in future studies the appliance will be re-deisgned to better protect the samples from dietary constituents while balancing the need for the dentine to be exposed to the oral environment to replicate in-vivo conditions. Participants could also be asked to keep a diet diary which might help explain why some samples fractured, and some dietary restrictions to avoid sample fracture could be implemented, however this would have the effect of making the situation less 'real life'.To the authors knowledge the Pashley model has not previously been modified to include in situ evaluation of hydrolytic conductance of dentine to determine permeability. In situ studies are a common investigational tool to bridge the gap between in vitro and in vivo analysis, allowing exposure to the oral environment whilst still maintaining a certain level of control of the specimen surface and more importantly allowing precise laboratory measurements to be recorded that cannot be achieved in vivo. Previous in situ studies performed to determine the potential of agents as occluding devices have used SEM scoring to determine the level of occlusion following exposure to the oral environment where a five scale scoring code is used to classify the surface from non occluded to fully occluded [22,34]. These type of studies are effective in differentiating the efficacy of occluding agents following treatment and exposure to the oral environment including the influence that an acid challenge has on the occluding agent. The limitations of this type of study however, are that the surface must be kept clean in order to collect reliable SEM images that show the degree of occlusion from the occluding agents rather than oral debris. In order for the appliance to remain clean, participants remove the appliances that house the dentine samples from their mouth during mealtimes. A second disadvantage of this study methodology is that tubule occlusion at a distance from the surface is not detected, demonstrating unsuitability of the method for the tubule occlusion characteristics of potassium oxalate. The modified Pashley cell model used in the present study allowed the participants to retain the appliances in their mouth whilst eating and drinking to more closely replicate the clinical situation, with images assessed from cross section of the discs rather than imaging from the surface only.

## 5. Conclusions

Treatment of patent dentine tubules with a potassium oxalate gel strip significantly reduced the rate of fluid flow through dentine discs. Modifying the Pashley cell model to include an in situ stage where treated and untreated dentine were housed in the oral environment for 14 days demonstrated further reduction of fluid flow through the dentine tubules. The latter reduction in fluid flow following oral exposure could be due to oral debris forming on the surface from the oral environment and dietary products as well as further formation of calcium oxalate within the dentine tubules due to saliva exposure. Given that there were no dietary restrictions it would appear that occlusion is resistant to dietary acid challenge. The novel technique at measuring hydrolytic conductance through dentine successfully demonstrated both efficacy and durability of the potassium oxalate strip at dentine tubule occlusion when challenged in the oral environment.

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**Figures**

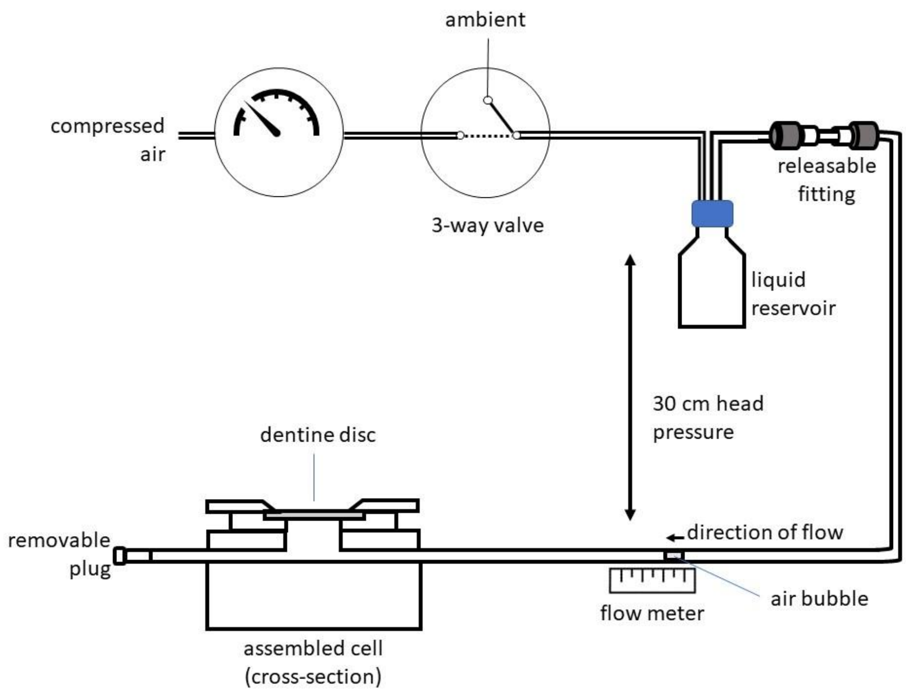


Figure1. A schematic of Pashley Flow Cell apparatus [24].

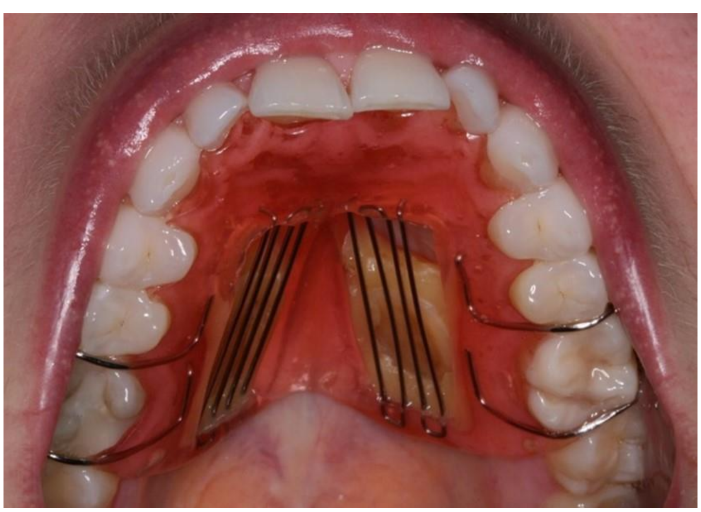

Figure 2. Palatal appliance housing two dentine discs

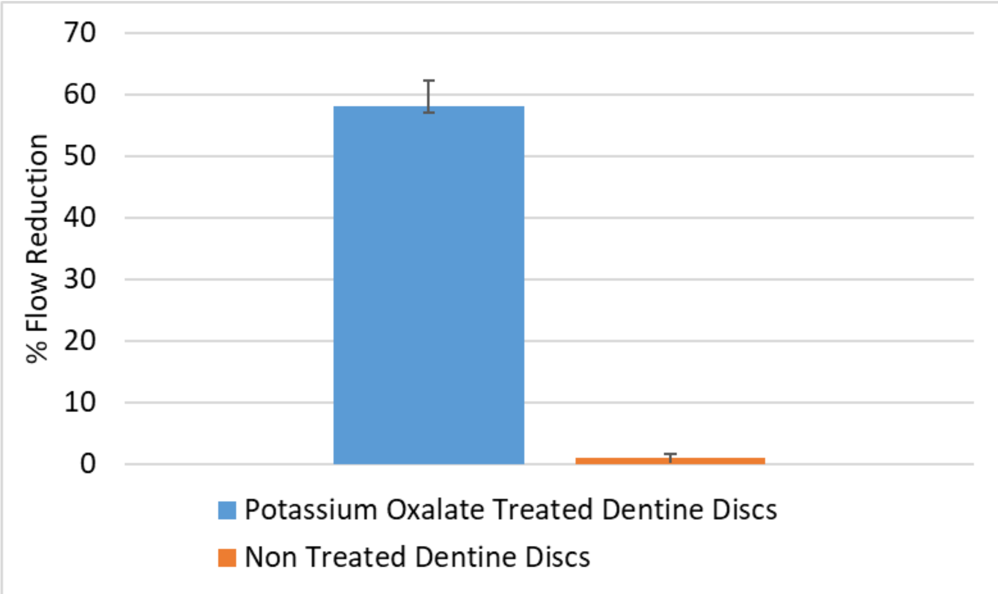


Figure 3. Fluid flow reduction through dentine discs following treatment with a potassium oxalate containing strip or following no strip treatment (0% reduction = maximium fluid flow, 100% reduction = no fluid flow).

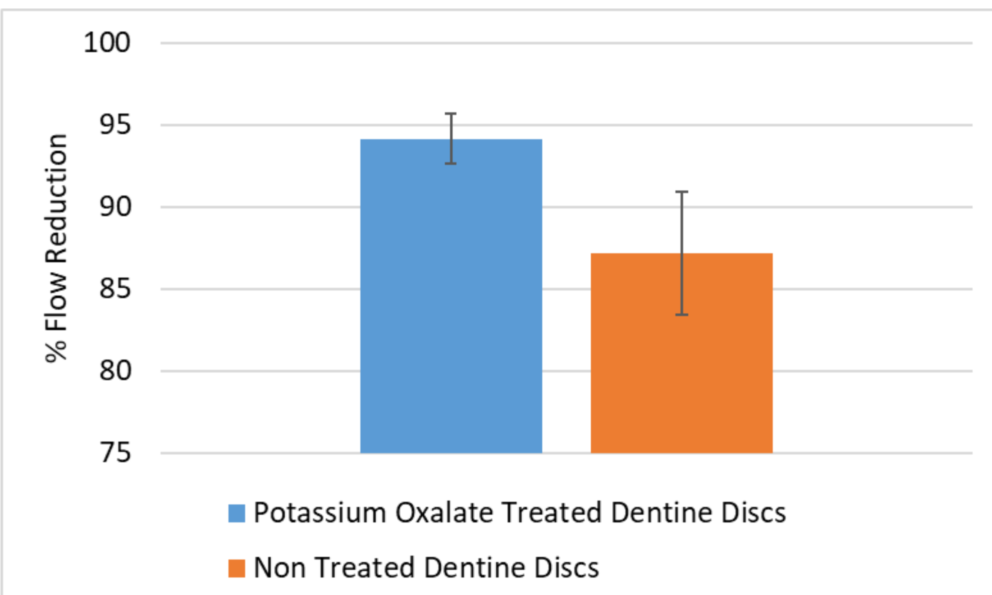


Figure 4: Amount of fluid flow reduction through dentine discs following treatment with and without potassium oxalate containing strip and 14 days wear in the oral cavity (0% reduction = maximium fluid flow, 100% reduction = no fluid flow).

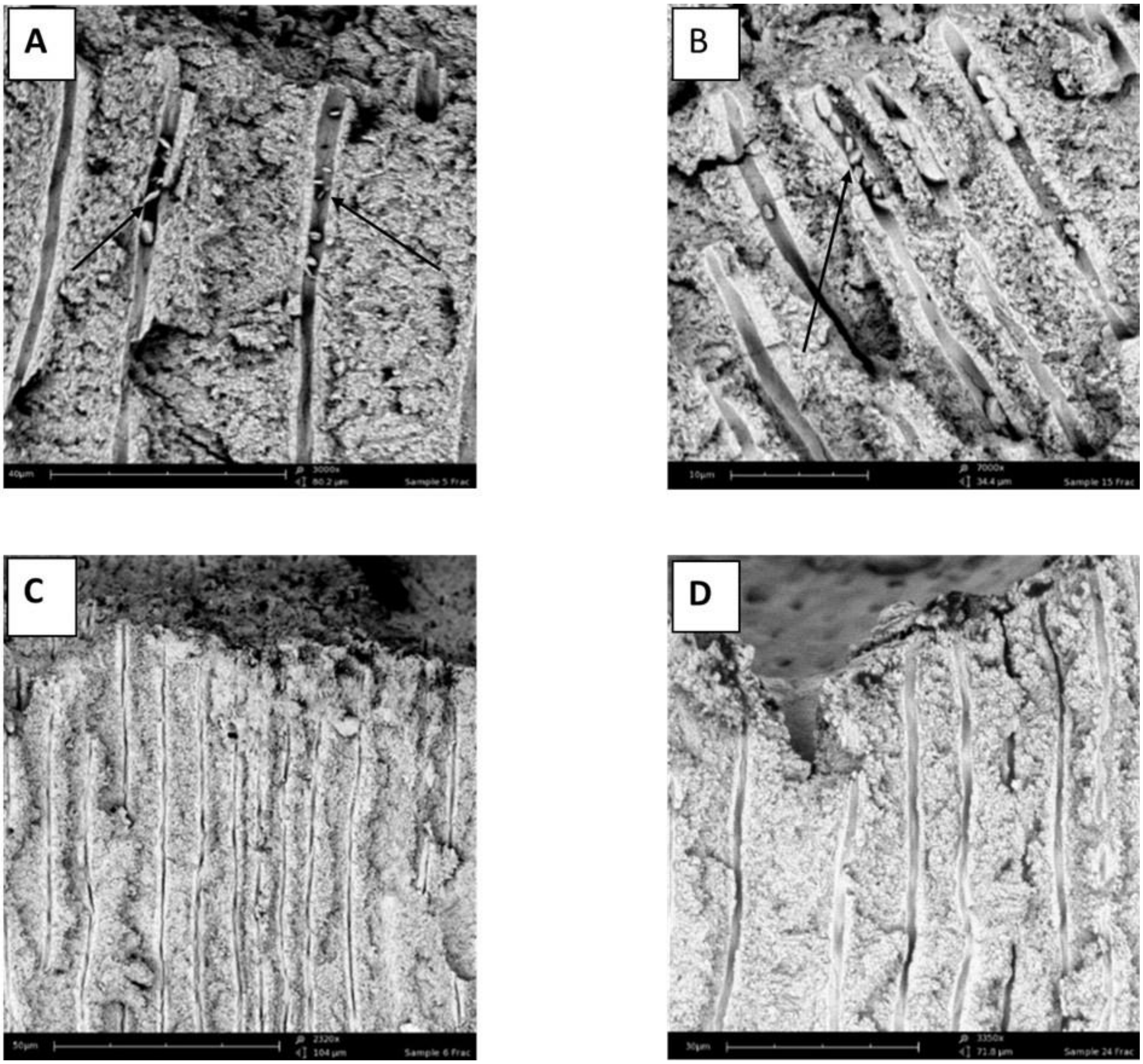


Figure 5: SEM images of cross sectional dentine discs following 14 days wear in the oral appliance. Oxalate particles are highlighted in the treated discs (A and B) whilst there are no particles visible in the non-treated discs (C and D).