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Hydraulic Conductance Model Optimisation for Assessing Dentifrice Dentine Occlusion

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Aims

It is widely known that dentine hypersensitivity can be relieved by either physical occlusion of the dentine tubules or through nerve depolarisation, and that this is achieved through brushing teeth with dentifrice containing the respective technologies[1]. A way of determining the occluding potential of a dentifrice is to determine the hydraulic conductance of dentine discs following treatment with the dentifrice[2]. Thus, the aim of this study was to:

- evaluate optimal dentine disc preparation conditions for the removal of smear layer formed as result of cutting discs prior to their use for hydraulic conductance (HC) studies
- develop a real-time *in vitro* hydraulic conductance model using a microfluidic cell, pressure controller, a digital flow sensor to accurately measure fluid flow through the dentine tubules

Methods

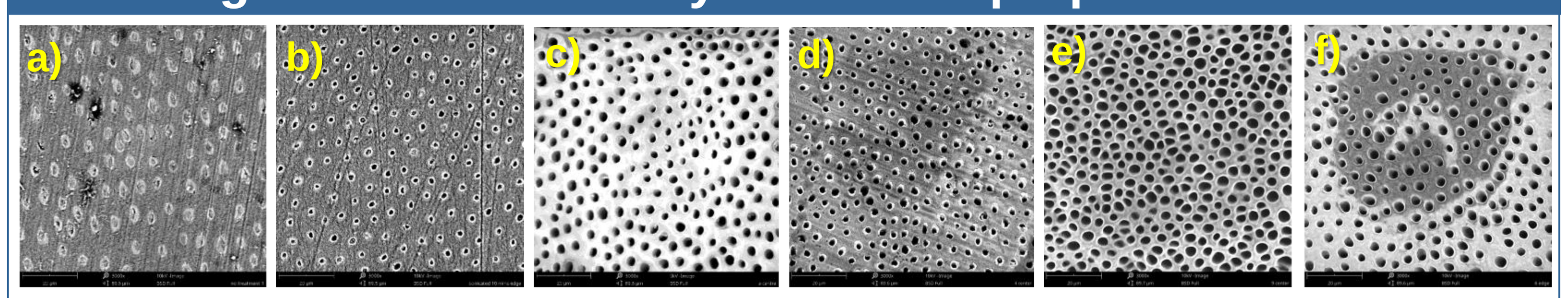
- Human dentine discs obtained from caries-free unrestored human molars were treated by varying the following parameters; type of acid (Ethylenediaminetetraacetic acid, EDTA vs Citric Acid), acid etching concentration (1% to 10%), type of agitation (shaking by hand vs sonication via ultrasonic water bath), acid etching duration (1 min vs 2 min) and sonication duration (2 min to 20 min) as well as the order of the preparation procedure (acid etching vs sonication) to evaluate smear layer removal.
- Visual characterisation of the dentine discs was performed using scanning electron microscopy (SEM) on the Desktop Phenom SEM by determining the number of occluded, partially occluded and non-occluded tubules on 3 images taken across the cross-section of 3 separate dentine discs. Each set of 3 discs were prepared with 14 different treatment conditions. Elemental analysis via energy dispersive spectroscopy (EDS) was also performed using the same equipment to determine efficiency of the smear layer removal under the various conditions. Confocal laser scanning microscopy (CLSM) was carried out on discs that had undergone 3 conditions (no treatment, 2 minute acid etch 1% w/w citric acid (shaking), 5 minute sonication and 5 minute sonication, 2 minute acid etch 1% w/w citric acid (Shaking)), using Rhodamin B dye to visualise the effect of preparation order on depth of non-occluded dentine tubules.
- A microfluidic flow controller pumped fluid at a controlled pressure of 70 mbar. A digital flow sensor enabled quantification of real-time flow rates of a near-physiological perfusion solution over the prepared dentine disc mounted in a microfluidic cell. A baseline flow rate reading over 5 minutes was recorded.
- Dentine discs were then brushed with Sensodyne toothpastes with occluding mode of action (via stannous fluoride or NovaMin) as positive controls and nerve-depolarising mode of action (via potassium nitrate) as negative control, in slurries (1:3 with artificial saliva) for 2 minutes via 4 successive brushing steps. This was followed by a 10% citric acid challenge to mimic acid found in diet and test the acid resistance of the toothpaste. This was then followed by 5% oxalic acid treatment for 5 min to fully occlude the dentine and verify the occluding potential of the model.
- The percentage reduction in flow rate after brushing was calculated to indicate the occluding efficacy of the dentifrices.

Results

Optimal Dentine Disc Preparation Condition

- Out of the 14 different preparation conditions, etching via shaking with 1% citric acid for 2 minutes followed by 10-minute sonication in deionised water (DI water), and thorough rinsing with DI water was found to give ~99.3% non-occluded tubules from SEM image analysis. 10% citric acid and acid etching via sonication were found to damage the disc as evident in Figure 1c and 1e and therefore were eliminated.

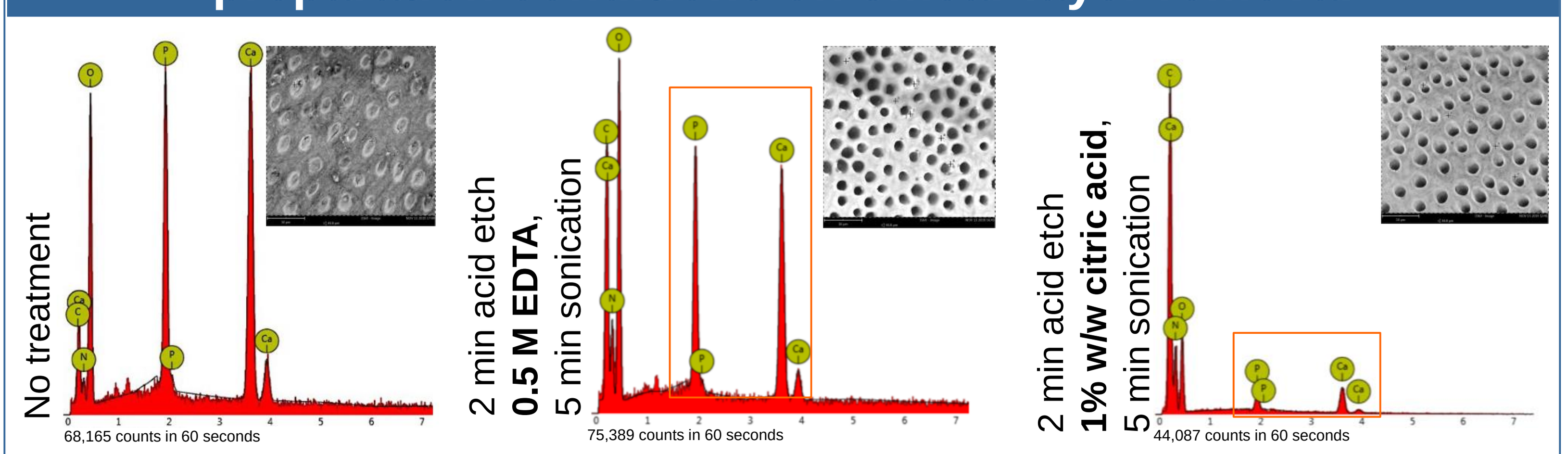
Figure 1. A selection of SEM micrographs of dentine discs following different smear layer removal preparation conditions



SEM micrographs at 10keV, 3000x magnification as acquired from Phenom SEM, n=3: a) No treatment, b) Sonicated in DI water for 10 min, c) Sonicated in DI water for 10 min, 2 min acid etch with 10% citric acid via shaking, d) 2 min acid etching with 0.5M EDTA via shaking followed by 5 min sonication in DI water, e) 2 min acid etching with 1% citric acid via sonication followed by 10 min sonication in DI water, and f) 2 min acid etching with 1% citric acid via shaking followed by 10 min sonication in DI water.

- Elemental analysis confirmed effective removal of smear layer with citric acid through reduction in the Ca and P peaks attributing to the hydroxyapatite of the smear layer.

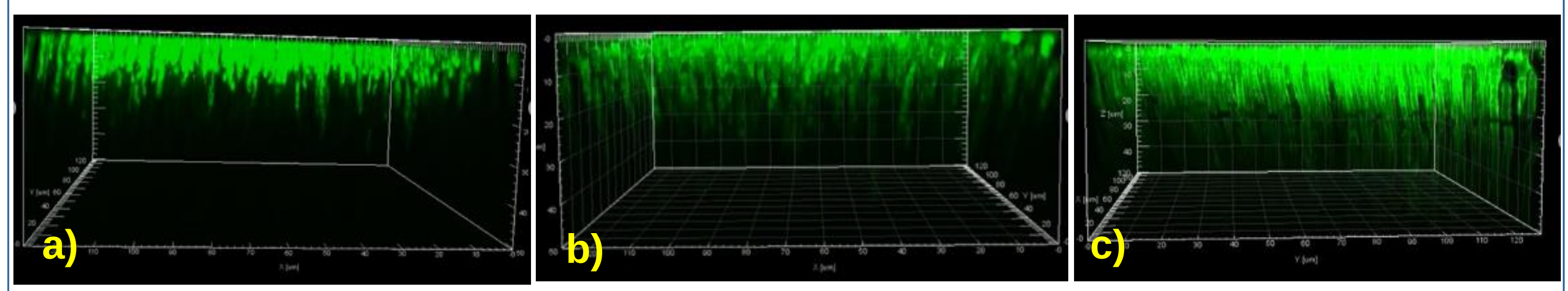
Figure 2. EDS spectra to determine effect of dentine disc preparation conditions on smear layer removal



EDS spectra acquired via Phenom SEM at 6000x magnification, n=3. The N, C and O is present in every image (attributed to the collagen matrix in dentine) whilst Ca and P reduced when etched with EDTA and citric acid, with citric acid having the greatest effect in reducing the Ca and P peaks and thus the solubilisation of the smear layer.

- CLSM indicated non-occluded tubules for up to 40µm depth when acid etching was performed before sonication of the disc thus confirming the more efficient order of preparation.

Figure 3. CLSM imaging to determine effect of order of sonication and acid etching on depth of smear layer removal

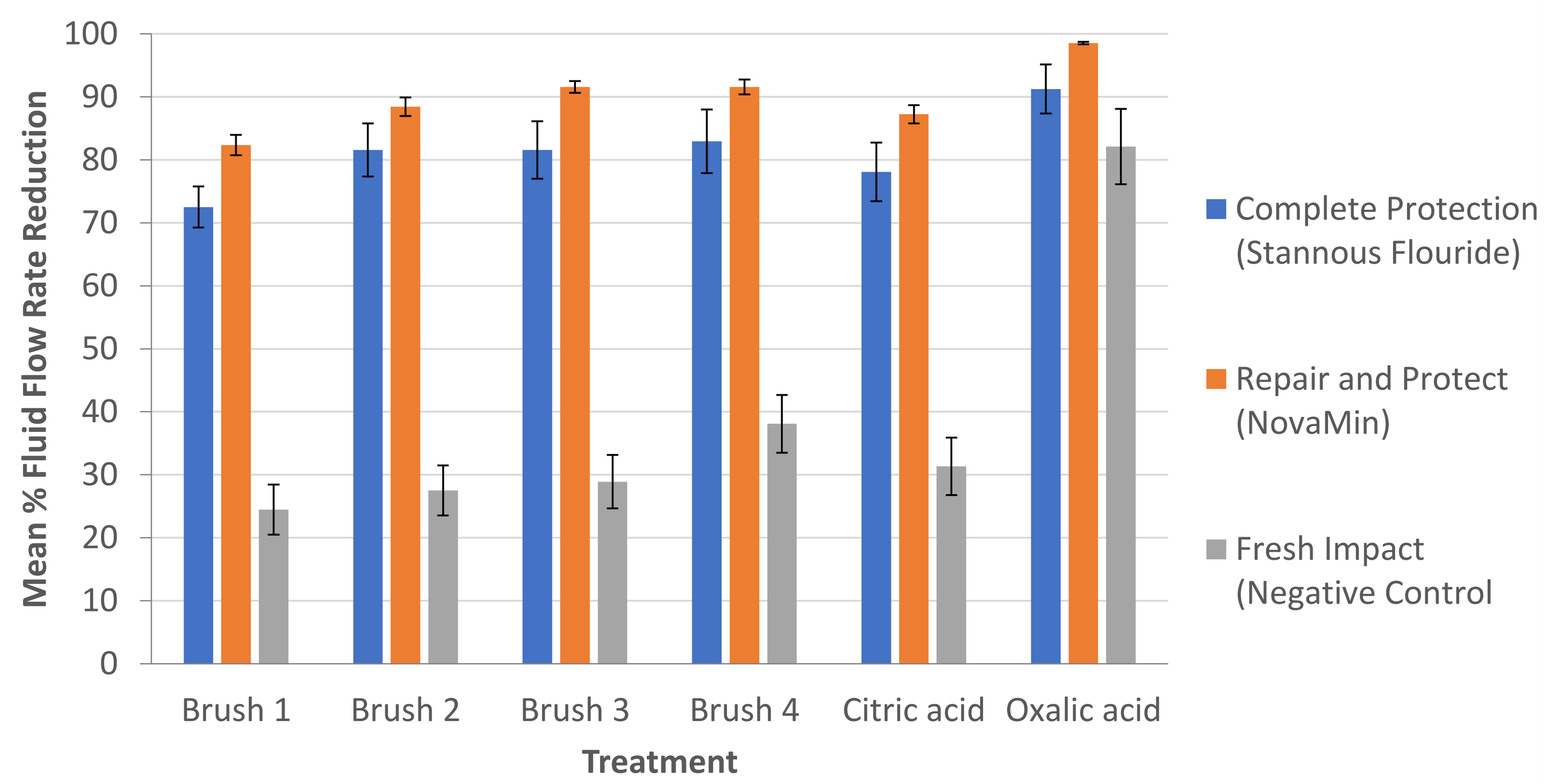


Confocal 3D stacked images down to 50µm, with tubules stained with 0.1mM Rhodamin B and imaged with Olympus Fluoview v4.10, n=3, a) No treatment (~10µm tubule depth), b) Sonication in DI water followed by acid etching with citric acid (~30µm tubule depth), c) Acid etching with citric acid followed by sonication in DI water (~40µm tubule depth).

Hydraulic Conductance of Dentine Disc

- Both the occluding toothpastes, Complete Protection and Repair & Protect, showed a mean percentage fluid flow reduction of over 80% at the 4th brushing whilst the non-occluding paste was below 40% thus confirming a significant difference between the positive controls and the negative control in their occluding capability. The reduction in flow rate observed with the non-occluding toothpaste can be attributed to the silica and sodium fluoride present in the formulation where latter causes the formation of relatively insoluble calcium fluoride crystals[3].

Figure 4. Hydraulic Conductance of Dentine Discs Treated with Various Dentifrice Technologies



The graph shows the effect of successive brushing of dentine disc with various toothpastes, on the perfusion solution flow rate. Higher mean percentage fluid flow rate reduction denotes better occluding capability of the toothpaste. 10% citric acid challenge treatment did not increase the fluid flow rate significantly and 5% oxalic acid treatment irreversibly occluded the tubules, n=15 discs per toothpaste

Conclusions

- The most effective smear layer removal preparation condition for dentine discs was identified to be acid etching first with 1% citric acid for 2 minutes to solubilise the Ca and P rich surface followed by 10-minute sonication in deionised water to dislodge the smear plugs and detach them from the peritubular dentine, and thorough rinsing with DI water to remove the residue.
- The positive controls (Sensodyne Complete Protection containing stannous fluoride and Sensodyne Repair & Protect containing NovaMin) were the most effective in reducing hydraulic conductance (i.e. dentine permeability) and split from the negative control (Sensodyne Fresh Impact containing potassium nitrate) as expected for the percentage reduction in fluid flow rate through the dentine disc.
- Acid challenge did not decrease the flow rate reduction by more than 10% thus confirming the acid resistance of the toothpastes.
- An *in vitro* model to determine occluding capability of dentifrices has thus been developed and may potentially be used to predict clinical performance of dentifrices developed for the treatment of dentine hypersensitivity.

References

1. Arnold WH, et al. *Journal of Dentistry*, 2015; Volume 43:440-449
2. Pashley DH, et al. *Journal of Periodontology*, 1984; Volume 55: 522-525
3. Pashley DH, *Journal of Endodontics,* 1986; Volume 12:465-474

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