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Dentin tubule occlusion by modified Novamin-containing dentifrices

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Aims

The widely accepted understanding of the physical occlusion of dentin tubules, providing relief from dentin hypersensitivity, has been well established over recent years[1]. Consequently, analytical techniques have been developed to characterise the formation and depth of occluding material on dentin, including the use of Focussed Ion Beam Scanning Electron Microscopy (FIB SEM)[2,3].

The aim of this study was to investigate, *in vitro*, the effect of modified Sensodyne Repair & Protect **(SRP)** dentifrices containing Novamin and either high surface area silica **(HSAS)** or standard abrasive silica **(SAS)** on dentine tubule occlusion. The extent of occlusion was measured using Hydraulic Conductance to determine the reduction in % fluid flow rate and FIB-SEM to measure occlusion depth and in-tubule distribution.

Methods

Whole human dentin discs ~800µm thick were polished flat, etched in 10% citric acid solution (pH3.75) for two mins, rinsed in deionised water and divided into three dentifrice treatment groups (n=10) :-

i). SRP + HSAS; ii). SRP + SAS; iii). SRP Control

For Hydraulic Conductance samples were mounted in a split cell and an initial baseline flow rate measurement was made for each treatment group disc. Dentin samples were then treated twice daily for five days by brushing with the test dentifrices (electric toothbrush; 1.1g +/- 0.1g of dentifrice; 200g downward force for 10 seconds) followed by resting in the resultant slurry for 30 seconds, rinsing with deionised water and incubation in 20ml of fresh artificial saliva at 37°C. Hydraulic Conductance measurements were made daily to assess the degree of fluid flow rate reduction by occlusion. After four days of treatment dentin samples were subjected to an acid challenge using proprietary grapefruit juice (Tesco Smooth – 1ml per minute flow for five minutes) followed by a final measurement (Day 5).

Following these measurements samples were removed from split cells and prepared for FIB-SEM analysis. An FEI Helios 650i FIB/SEM instrument fitted with an Oxford Instruments 150 EDS detector was used with a 2keV, 200pA beam current in both secondary electron (SE) and back-scattered (BS) detection modes. For FIB-sectioning, ion milling was carried out with a 9nA beam current at 30keV for bulk removal of material.

Samples for SEM were gold-coated followed by cleaving, to investigate dentin tubule occlusion through the bulk material. To examine occlusion in the near-surface (throat) regions of tubules samples were FIB-sectioned and Pt coated prior to SEM analysis. Additional SEM imaging was carried out on the treated dentin surfaces to visibly assess “top-down” tubule occlusion.

Results

Hydraulic Conductance

The results of hydraulic conductance measurements on the three treatment groups over a five day period are summarised in fig. 1.

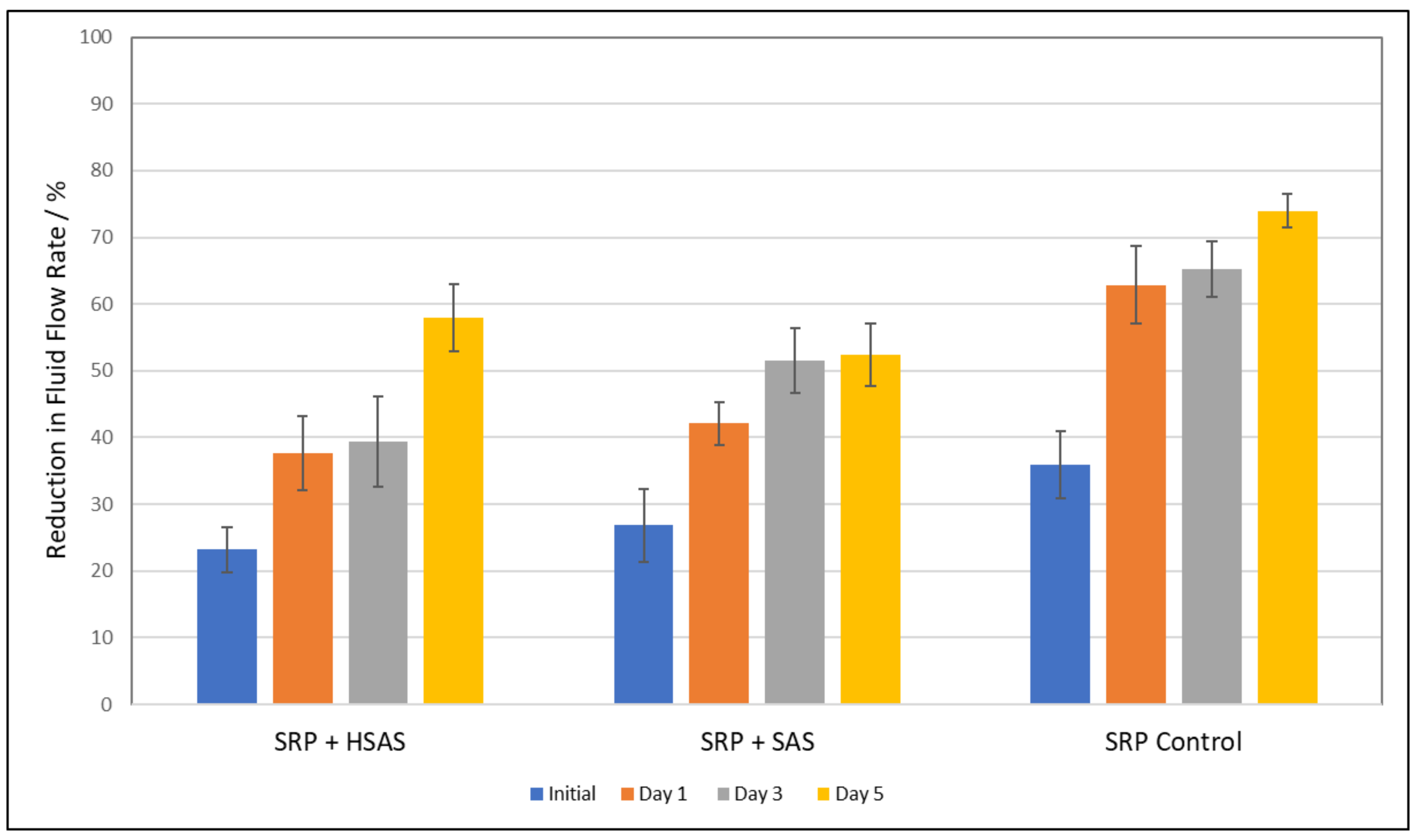


Figure 1. Hydraulic Conductance measurements of the reduction in fluid flow rate % with treatment

Data was statistically evaluated by one-way ANOVA and Tukey means comparison. All data sets were shown to be normally distributed, with probability factors >0.05, using the Shapiro-Wilks test.

All three groups showed a reduction in fluid flow with progressive treatment and would therefore provide a level of tubule occlusion.

SRP Control showed a statistically higher level of fluid flow reduction on day 1 c.f. SRP + HSAS and SRP + SAS and was directionally higher on days 3 and 5. SRP + SAS was directionally higher in fluid flow reduction c.f. SRP + HSAS on days 1 and 3 but lower on day 5.

FIB-SEM – Occlusion in tubule throat regions

SEM analysis of FIB-sectioned samples showed wide variations in tubule occlusion in throat regions. To obtain representative mean values of occlusion depth >100 tubules were examined per group. Typically, occluded material penetrated to a mean depth of ~3µm – 5µm, with both SRP + HSAS and SRP Control having comparable values which were directionally higher than SRP + SAS. Example FIB-SEM images are shown in fig. 2.

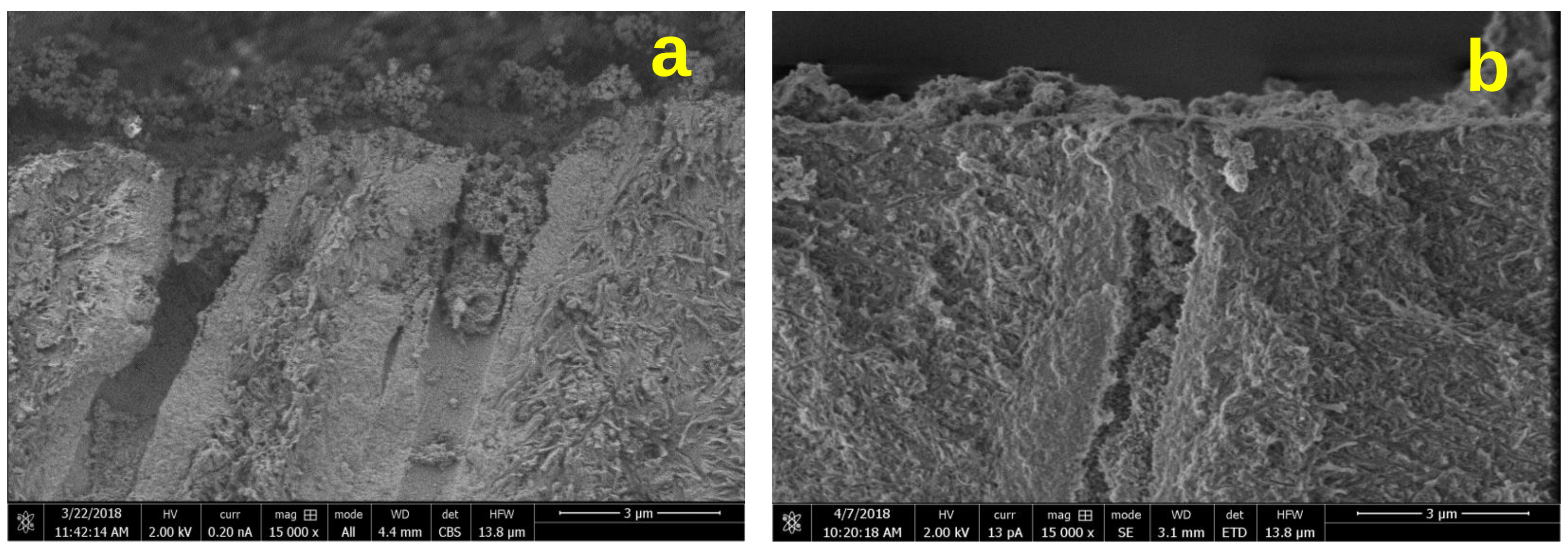
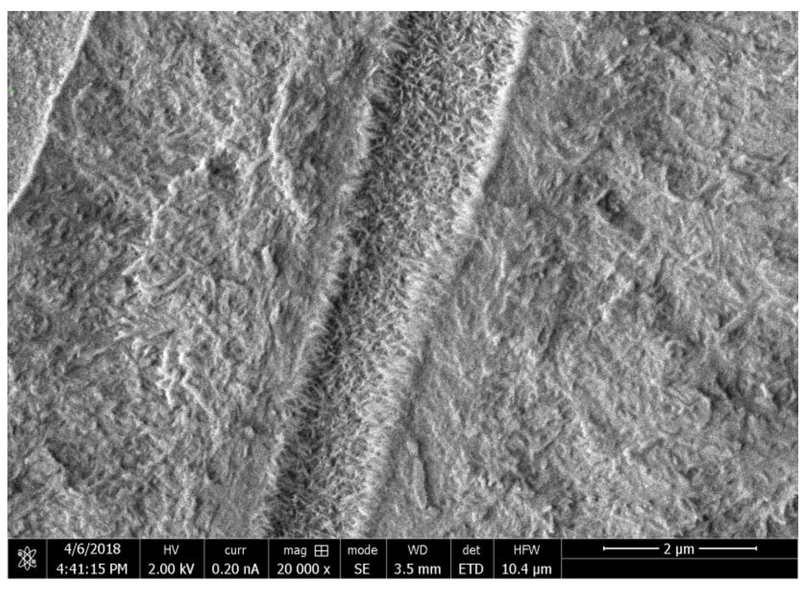


Figure 2. Example SEM images of FIB-sectioned samples – SRP + HSAS (a); SRP Control(b)

SEM Imaging of cleaved samples

Cleaved cross-sections of samples imaged at 50µm below the treated surface showed some evidence for occlusion within tubules in all groups. Where present, the tubule filling at this depth consisted of amorphous textured material with some areas exhibiting elongated crystals within peritubular sidewalls.



Treatment group SRP + HSAS showed some evidence for higher levels of filled tubules at 50µm depth compared to SRP + SAS and SRP Control.

Fig. 3 shows the crystalline “filled” appearance of a tubule from Treatment Group SRP Control at 50µm depth.

Figure 3. SEM image - cleaved section SRP Control - 50µm depth

SEM Imaging of Surface Features

Secondary electron images of the surface of treated samples (fig. 4) revealed top-down tubule occlusion on all treatment groups. Wide variation in surface coverage and occlusion was noted within all groups. Occluding material in SRP + HSAS tended to protrude from tubules indicating a possible higher level of adhesion to the collagen matrix. The level of occlusion appeared to be higher for SRP + HSAS and SRP Control c.f. SRP + SAS.

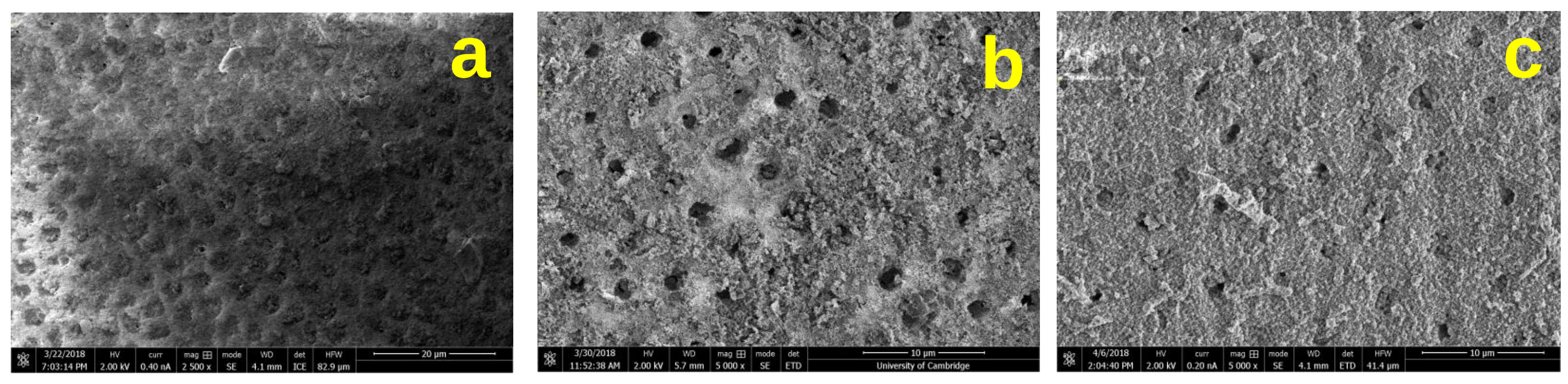


Figure 4. SEM images of treated surfaces :- SRP + HSAS (a); SRP + SAS (b) and SRP Control (c)

Conclusions

This in vitro study of dentin tubule occlusion by modified Novamin-containing dentifrices has shown :-

- Directionally higher reduction in fluid flow rates over five days for SRP Control treatment group c.f. SRP + HSAS and SRP + SAS.
- Occlusion depths in tubule throat regions of 3µm - 5µm, with comparable mean values for SRP + HSAS and SRP Control, both directionally higher than SRP + SAS.
- Some evidence for filling of tubules with amorphous and some crystalline occluding material within peritubular sidewalls at around 50µm depth, in all groups.
- Top-down occlusion visible on dentin from all treatment groups with higher occlusion for SRP + HSAS and SRP Control c.f. SRP + SAS.

References

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