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Short-term response of three resin-based materials as desensitizing agents under oral environmental exposure

EGLE MILIA[1], GIORGIO CASTELLI[1], ANTONELLA BORTONE[1], GIOVANNI SOTGIU[2], ANDREA MANUNTA[1], ROBERTO PINNA[1] & GIUSEPPE GALLINA[3]

[1]*Department of Surgery, Microsurgery and Medicine,* [2]*Department of Biomedical Sciences, University of Sassari, Italy, and* [3]*Department of Dental Science, University of Palermo, Palermo, Italy*

Abstract

Objective. This paper focuses on clinical responses after 7 days of oral exposure to two resin-based materials as desensitizing agents compared to a fluoride varnish and on morphological and analytical study as a means to elucidate the mechanism of action. ***Materials and methods.*** The elemental composition of Vertise™ Flow (VF), Universal Dentine Sealant (UDS) and Flor-Opal® Varnish (FOV) were investigated by using an X-ray energy dispersive spectrometer (EDX) in conjunction with a scanning electron microscope (SEM). SEM morphology of the material-treated dentine surfaces and pain reduction ability according to the Visual Analogue Scale (VAS) were evaluated in selected hypersensitive teeth. Post treatments and 7 day controls were recorded with SEM and VAS measurements. Clinical data was analysed with the Student's *t*-test for paired data, with a 5% significance level. ***Results.*** Silicon, ytterbium and alumina were the most present elements in VF, whilst calcium, chloride, silicon and alumina were highest in UDS. Within a 7 day oral environment all the tested materials modified the treated-dentine surfaces showing tubular occlusion of different morphology. Clinically, the efficacy of all materials was similar after a 7-day examination. However, VAS scores were significantly reduced if compared with the baseline ($p < 0.05$). ***Conclusions.*** Within the limits of this study, data indicate that both resins are effective in sealing tubules and reducing VAS. A resin-related effect on the dentine's morphology was observed, which may influence the long-term response of the resins in the treatment of dental hypersensitivity, which requires further investigation.

Key Words: *adhesive resins, dentine hypersensitivity, fluoride, ultrastructure, VAS*

Introduction

Dentine hypersensitivity (DH) is a common and painful syndrome existing within 4–74% of the adult population [1]. DH is characterized by a short and sharp sensation of pain arising from the tubular dentine exposure to the oral environment as a result of enamel loss and/or gingival root surface exposure due to attrition, abrasion, erosion, abfraction or gingival recession [2]. The most widely accepted mechanism of DH is Brännström's [2] hydrodynamic theory, whereby thermal, drying, tactile or chemical stimuli promote fluid shifts in the exposed dentinal tubules, causing pain by activation of the pulp nerves [3].

Therefore, the occlusion of the tubules by different materials may reduce the fluid movement inside the dentinal tubules and the clinical symptoms of DH [4].

Thus, the efficacy of desensitizing materials has been evaluated by direct measurement of fluid flow through dentine, or dentine permeability, using *in vitro* fluid filtration systems [4–9] or an *in vivo* Visual Analogue Scale (VAS) measurement of pain [10–14] and has been correlated with various stimuli that induce pain in the exposed dentine [15]. Furthermore, the material-treated dentine surfaces have been investigated using a scanning electron microscope (SEM) [6,16]. However, when the occlusion of the tubules was superficial or not adherent to the tubular wall, daily tooth brushing, saliva or consumption of acidic beverages easily opened the dentinal tubules, leading to short-term desensitization effects.

Some treatments of DH employ inorganic biomaterials [15–19] and organic biomaterials or resin-based materials [20–23].

Correspondence: Egle Milia, Viale San Pietro 43/c, 07100 Sassari, Italy. Tel: +0039 079 228437. Fax: +0039 079 272645. E-mail: emilia@uniss.it

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The binding quality of biomaterials influences DH treatment outcomes, as biomaterials can bind to the exposed dentine surface and within the openings of the dentinal tubules to mediate the formation of a tight seal [24–27]. *In vitro* studies recently showed stable tubular occlusion through the use of calcium silicate cements [16], which are largely used as bioactive materials in dentistry. However, the clinical use of calcium silicate cements (i.e. mineral trioxide aggregate (MTA) and Portland Cement (PC)) is limited, due to the long setting time entailing possible oral interferences with bio-activity capacity [28]. Thus, selected accelerants (i.e. calcium chloride) have been suggested to accelerate the setting of MTA and PC, increasing their acceptance in wider clinical situations [29–32].

With regard to the resin-based materials, the desensitizing effect has been attributed to the formation of a resin seal in the exposed dentine with tubular occlusions by resin plugs [9,23,31]. Nevertheless, different data are reported when resin-based materials were used in the treatment of DH, which may reflect different approaches and reactivities of resins [7,9,23,31] and a possible degradation of the polymer matrix in an oral environment [32,33]. Unfortunately, objective and comparative research on resin-based materials is often hindered by the scarcity of specific information about some of their chemical components, such as the proprietary monomers. Descriptive terms are often used to indicate the working mechanism avoiding the disclosure of active ingredients in the resin matrix. Even if some resin properties may be deducted through chemical analysis [32,34] it is paramount to know their behaviours in oral environment conditions [10,27,29,30].

In light of the considerations above and of the scarcity of resin-based materials indicated to treat DH, we decided to conduct both an elemental analysis and a morphological and clinical assessment of the efficacy of Vertise™ Flow (VF) (Kerr Corporation, Orange, CA), a self-adhering resin composite, that is suggested for DH treatment, and Universal Dentine Sealant (UDS) (Ultradent, South Jordan, UT), a desensitizing resin sealant, whilst controlling for the influence of the oral environment which can trigger different responses. As a control group we used a fluoride-containing varnish.

The null-hypothesis was that the three resins will not reduce the VAS measurement of pain, either initially or after 7 days of exposure to oral fluids.

Materials and methods

VF is a proprietary self-adhering flowable resin composite. Wej et al. [33] recently reported that VF included an organic matrix of glycerol phosphate dimethacrylate (GPDM), proprietary methacrylate co-monomers, a filler of pre-polymerized particles of barium glass, nano-sized colloidal silica and nano-sized ytterbium fluoride.

UDS is described by the manufacturer as a biocompatible, non-polymerizable, high molecular weight proprietary resin sealant in an alcohol solvent.

In this study Flor-Opal® Varnish (FOV) (Ultradent, South Jordan, UT), a 5% sodium fluoride (NaF) varnish, was used as a control group due to the ability of fluoride to react with calcium ions in dentinal fluid to produce tubular occlusion by insoluble CaF_2 crystals [6,20,21] with dentine permeability reduction [10,23,35–37]. (Table I shows the components and modes of application of the materials tested in this study).

Elemental analysis

The elemental composition of VF, UDS and FOV was investigated using an X-ray energy dispersive spectrometer (EDX) (INCA-X-acta, Oxford Instruments, Tubney Woods, Abingdon, Oxfordshire, UK) in conjunction with an environmental scanning electron microscope (ESEM) (EVO® LS 25, Zeiss, Oberkochen, Germany). EDX was carried out using an accelerating voltage of 20 kV and ESEM was used for imaging of each sample at standardized magnification (200×, 1000×).

For the semi-quantitative X-ray analysis, VF, UDS and FOV (0.5 mL) were weighed, placed in a thin layer over Perspex® slabs mounted on aluminum stubs (Agar Scientific, Stansted, UK). Three stubs were made for each tested material and the analysis was performed twice for each sample. The elemental analysis (weight percentage and atomic percentage) was performed in low-vacuum conditions (20 Pa). Atomic number, absorption and fluorescence corrections were applied during the analysis with the ZAF correction method.

Experimental design

Subjects who had hypersensitive teeth were selected from an ongoing programme evaluating desensitizing agents at the Dental Clinic of the University of Sassari. Two clinicians selected patients complaining about hypersensitivity and who had reported this to the Department of Periodontology at the Dental Clinic. The protocol and informed consent forms were approved by the ethics committee at the University of Sassari (n° 1000/CE). The medical and dental history of the patients was collected and sensitive teeth were differentiated from other clinical conditions which frequently interfere with DH. All the subjects were thoroughly informed about the study's purpose, risks and benefits. A total of 86 patients with hypersensitive teeth were collected after an intake period of 8 months. The study inclusion/exclusion criteria were the following: (1) patients

were considered suitable for the study if they had sensitive teeth showing abrasion, erosion or recession with the exposure of the cervical dentine; (2) teeth with subjective or objective evidence of carious lesions, pulpitis, restorations, premature contact, cracked enamel, active periapical infection or which had received periodontal surgery or root-planning up to 6 months prior to the investigation were excluded from the study. Other exclusion criteria were professional desensitizing therapy during the previous 3 months or use of desensitizing toothpaste in the last 6 weeks. Patients were also excluded if they were under significant medication that could have interfered with pain perception (e.g. antidepressants, anti-inflammatory drugs, sedatives and muscle relaxants). As a consequence, the total study population included in the programme was of 74 subjects, 43 female and 31 male, aged 27–75 years (mean age ± standard deviation: 53 ± 7 years) with a total of 286 hypersensitive teeth (mean teeth for patient 2 ± 1). The level of sensitivity experienced by the patient was considered as independent of the position of the hypersensitive tooth in the oral cavity [12].

Morphological study

VF, UDS and FOV's ability to occlude dentine tubules and their morphology on dentinal surfaces were evaluated in 30 selected patients, 18 female and 12 male, part of the total sample of 74 subjects with 30 hypersensitive teeth. Patients had 30 hypersensitivity teeth (11 premolars, 13 incisors, six cuspids), whose Grade III mobility and significantly reduced response to periodontal treatment suggested the need for extraction [38,39].

A full medical and dental history was taken and all the teeth were carefully examined to confirm the diagnosis of DH. The nature and scope of the study was explained and informed consent was obtained.

A week before treatment, patients received oral prophylaxis and were randomly assigned to three experimental groups (n = 10 per group). The treatments were carried out at random by one of the clinicians, while the other assisted. The teeth were isolated with cotton rolls and the treatment with VF, UDS and FOV was performed as summarized in Table I. As recommended, a halogen curing light (Optilux 501, Kerr Corporation, Orange, CA, USA; 11 mm exit window) under the standard curing mode (output wavelength range: 400–505 nm; output irradiance: 580–700 mW/cm^2) was used to allow light curing of VF. After the treatment, teeth were immediately extracted (n = 5 per sub-group) in sub-group 1 and after 7 days post-treatment (n = 5 per sub-group) in sub-group 2.

After extraction, samples were rinsed with distilled water at 37°C and fixed in a solution of 2.5% glutaraldehyde in 0.1 M PBS buffer (pH 7.2) for 72 h. In each sample, the treated cervical dentine was sectioned from the remaining crown and roots of the tooth with a water-cooled saw (Isomet low-speed saw; Buehler, Lake Bluff, IL) and then fractured into two halves in order to analyse the buccal surface and the longitudinal surface of the material-treated dentine surfaces. Samples were post-fixed in 1% osmium tetroxide, dehydrated in increasing concentrations of acetone (25–100%), dried by critical point drying and metal-coated. Specimens were then observed using a scanning electron microscope (SEM) (Zeiss, DSM 962, Oberkochen, Germany). Observations were recorded at standardized magnifications (1000×, 3000×, 5000×).

Clinical study

The study population consisted of another 36 patients, 19 females and 17 males, who were randomly selected from the total population of 74 subjects who had hypersensitive teeth. A total of 90 teeth (30 premolars, 44 incisors and 16 cuspids) constituted the group of hypersensitive teeth for the clinical effectiveness of VF, UDS and FOV.

A week before the experiment, patients received oral prophylaxis. Non-fluoride toothpaste, soft toothbrush and oral hygiene instructions were also provided in order to have standardized habits during the period of the study.

Table I. Desensitizing agents used in the study (manufacturer's data).

Code	Material	Manufacturer	Components	Batch no.	Mode of application
VF	Vertise™ Flow	Kerr Corporation (Orange, CA)	GPDM, methacrylate monomers, barium glass, silica, ytterbium fluoride*	122005	apply flow on a thin layer, scrubbing for 20 s, gently air-dry for 20 s, light cure 10 s
UDS	Universal Dentine Sealant	Ultradent (South Jordan, UT)	resin, alcohol	052809	Brush 30 s, paint a thin layer and gently air-dry for 5–10 s, saliva contact
FOV	Flor-Opal® Varnish	Ultradent (South Jordan, UT)	natural resin, sodium fluoride	122005	Brush 30 s, apply a smooth layer, scrubbing for 5–10 s, saliva contact

GPDM, glycerol phosphate dimethacrylate.
* [33].

Teeth were randomly assigned to three groups (n = 30 per group) for the treatment with the three desensitizing agents (Table I). At the baseline visit, they were reassessed for dentine hypersensitivity using the Visual Analogue Scores (VAS) of pain. Treatment was performed by one examiner, while the pain stimulus was given by the other examiner with the same equipment yielding similar air pressure each time.

The VAS scale consisted of a horizontal line that was 100 mm long, on which 'no pain' was marked on the right-hand extremity and 'unbearable pain' on the other. The patients expressed the intensity of the pain experienced by placing a mark at any point along the continuum. The distance, expressed in millimetres, from the right edge of 'no pain' was used as the VAS score. Each patient was asked to rate the perception of discomfort after the application of air via a dental syringe at 45–60 psi, 1 cm at the cervical third of the tooth after removing supragingival plaque with a low-speed handpiece with pumice powder and without fluoride. The adjacent teeth were covered by cotton rolls. The stimulus was delivered until reaction or up to a maximum duration of 10 s by the same examiner with the same equipment yielding similar air pressure each time. The subject's response was considered as the baseline measurement (PRE-1) mean ± standard deviation VAS score: 5.3 ± 2.1. Before the application of the material (PRE-1), immediately after (POST-1) and after 7 days of oral environment (POST- 2), the same clinician carried out the sensitivity test.

To compare the efficacy of the treatments, teeth were evaluated as a statistical unit rather than a subject. Data were elaborated using parametric tests (ANOVA for more than two samples adjusted according to Sidak's multiple testing) with a 5% significance level.

Figure 1 summarizes the experimental design used for the SEM morphological study and the clinical study in order to test different desensitizing materials.

Results

Elemental analysis

VF treatment left a layer of highly visible randomly distributed 5–40 μm particles (Figure 2). Spectra of silicion (Si), ytterbium (Yb) and alumina (Al) were highest in the layer in which also phosphorus (P),

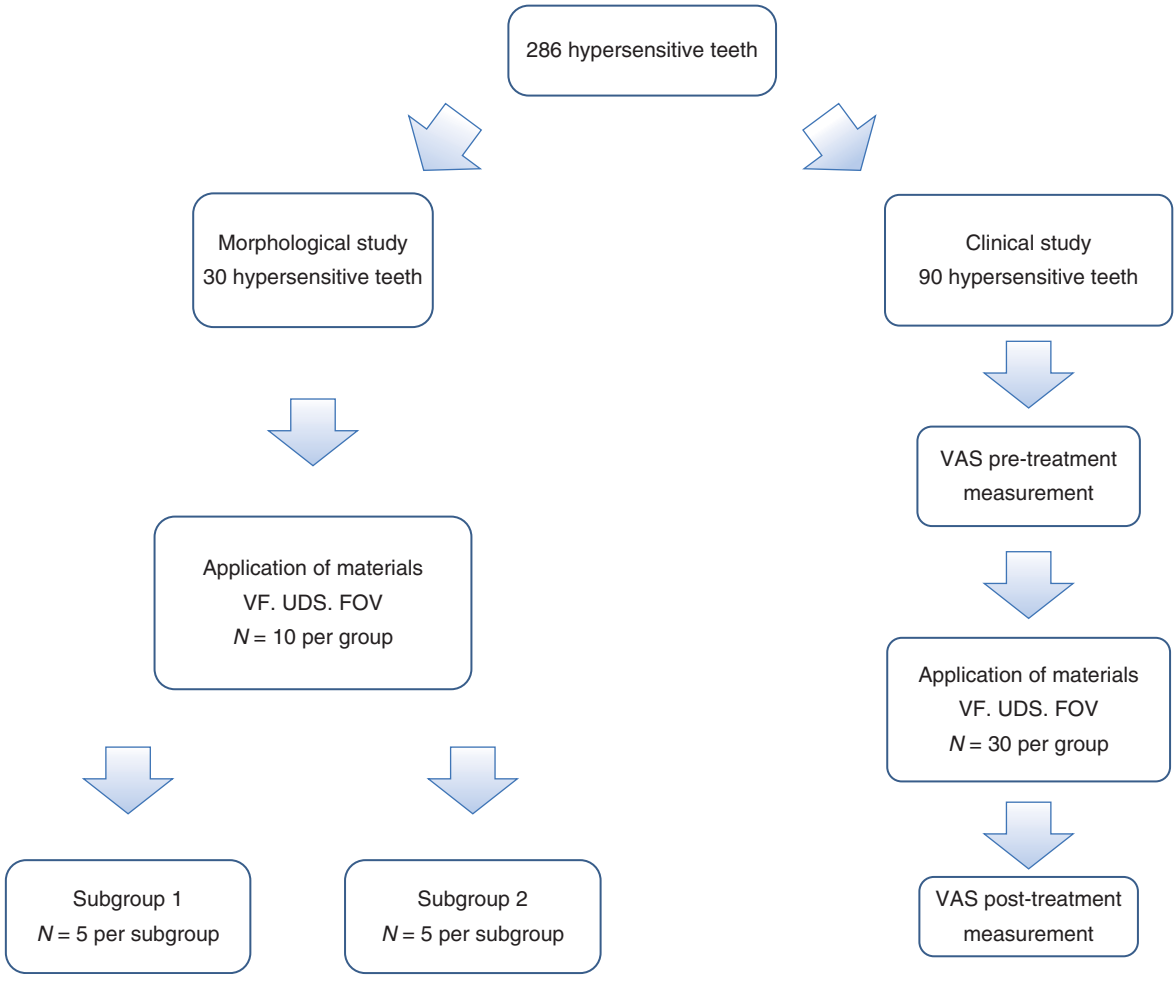

Figure 1. Summary of the experimental design to collect hypersensitive teeth and test different desensitising materials for the SEM morphological study and the clinical study.

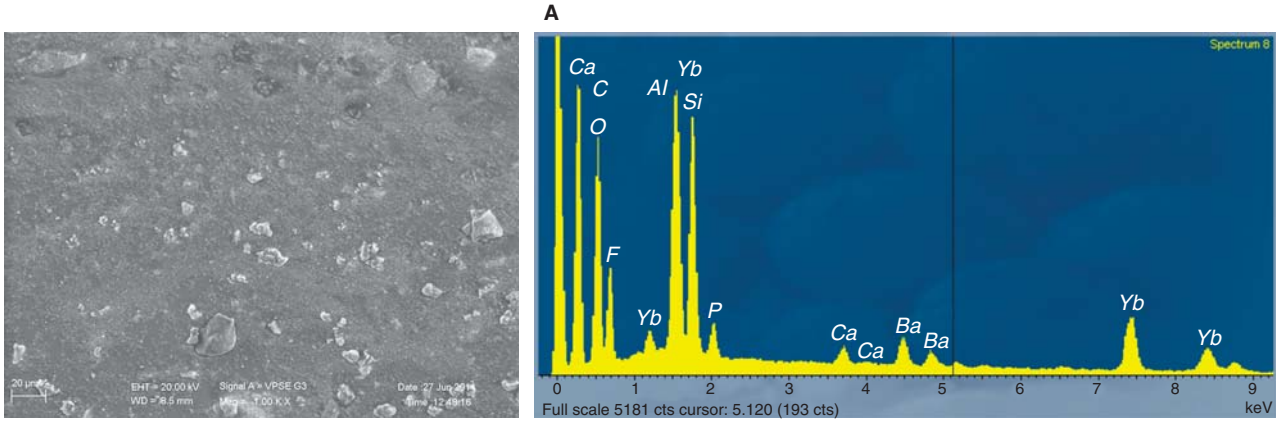

Figure 2. EDX analysis of Vertise™ Flow self-etching composite showing the ESEM morphological aspect of the self-etching composite composed by an amorphous matrix of nano-particles and highly visible randomly distributed 5–40 µm particles. EDX analysis in (A) reveals Si, Yb and F as the most represented elements in the matrix in which Al, P, Ca and Ba are also present.

calcium (Ca), barium (Ba) and fluoride (F) were found (Figure 2A).

UDS treatment left fine, dispersed particles of ~ 0.5 µm in a thin and smooth layer (Figure 3). Spots on these particles showed very high peaks of Ca and chlorine (Cl) (Figure 3A). The semi-quantitative analysis obtained by scanning different areas of the matrix highlighted Ca and Cl associated with Si and other oxides of Al, iron (Fe), chrome (Cr), potassium (K), sulphur (S), magnesium (Mg), titanium (Ti) and zinc (Zn) (Figure 3B).

FOV-treated samples showed a layer of particles embedded in a smooth matrix (Figure 4) rich in sodium (Na) and F peaks and with traces of Si and P (Figure 4A).

Morphological study

On the surface of the exposed dentine (ED) to the oral fluids, VF formed a thick, irregular coat that completely masked the underlying tubular dentine (Figures 5A and B). Cracks were also noted in ED. Longitudinal sections showed a coating ~ 3 µm thick composed of a matrix with crystal-like particles of different sizes. Tubule orifices were tightly blocked by the material and plugs of resin-like material were found inside the tubules (Figure 5C). After 7 days of exposure to the oral environment (sub-group 2), tubular orifices were still not visible on ED treated dentin surface, which showed cracks and gap formations (Figure 5D). Crystal-like precipitates were

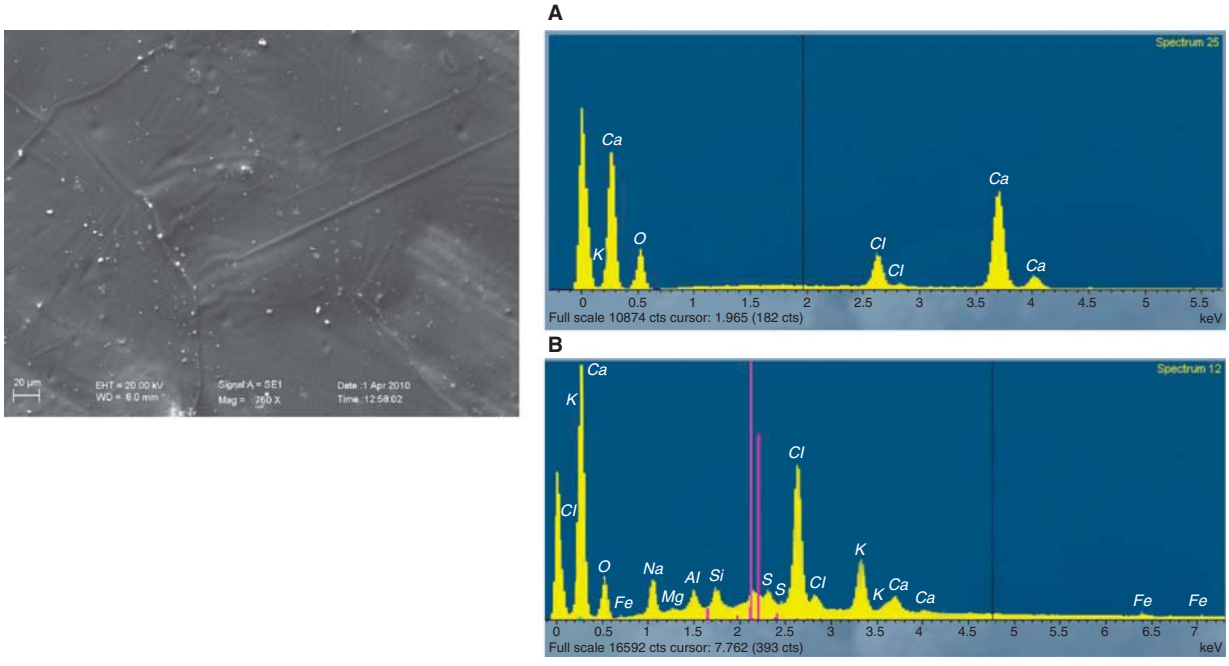

Figure 3. EDX analysis of Universal Dentin Sealant showing the ESEM morphology of the sealant composed by a smooth matrix with dispersed particles of ~ 0.5 µm. (A) EDX composition of the particles with very high peaks of Ca and Cl and (B) the semi-quantitative analysis obtained by scanning different areas in the matrix evidencing Ca and Cl peaks associated to Si and Al peaks as well as traces of Fe, Cr, K, S, Mg, Ti and Zn.

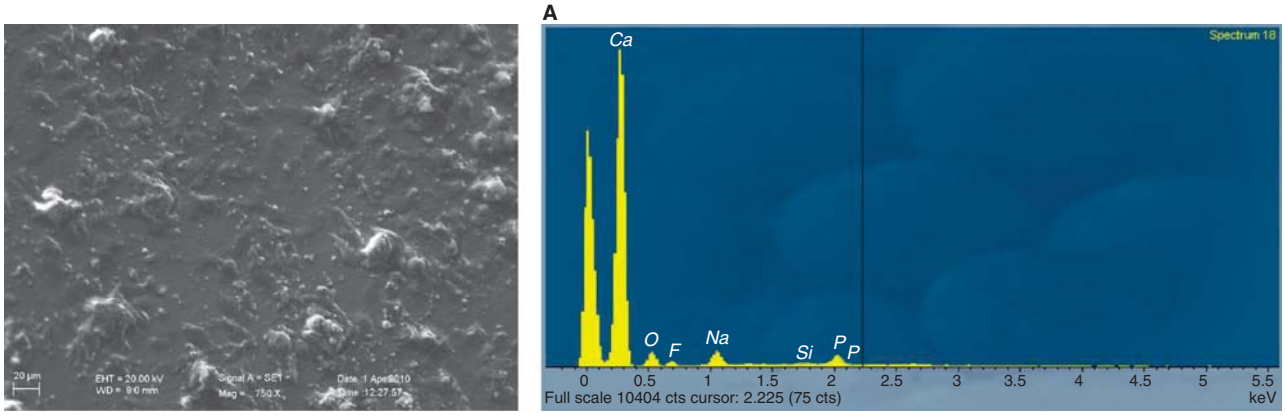

Figure 4. EDX analysis of Flor-Opal® Varnish showing the ESEM morphology of amorphous layer with particles and (A) the semi-quantitative analysis identifying Na and F as the main elemental components. Si and P are also retrieved in traces in the varnish.

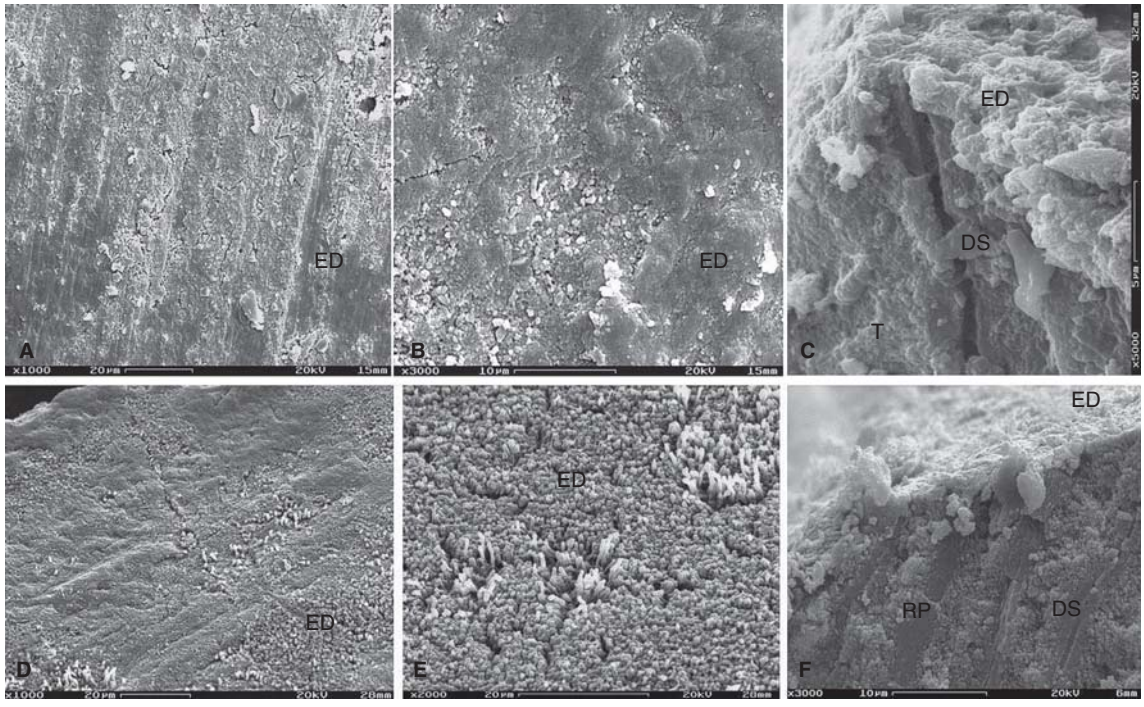

Figure 5. Representative SEM micrographs of Vertise™ Flow self-adhering composite immediately after application on the dentine (A, B) and after environmental exposure (D, E). (A and B) Two images at different magnifications of the dense, irregular layer containing particles masking the tubular dentine and showing cracks which could be caused by dehydration of the samples during the preparation procedure for the SEM. Longitudinal sections of exposed dentine (C) show the 3 μm thick coating with crystal-like filler particles of Vertise™ Flow. An interdiffusion layer of the self-adhering resin composite in the dentine cannot be disclosed by SEM under the standardized magnifications used in this study. Environmental exposure in (D) and (E) shows the cracks, which, compared with pre-aged images in (A) and (B), appear wider on the dentine surface, along with gaps. Crystal-like particles were also observed on the exposed surface. Longitudinal sections of exposed dentine (F) show the tubular occlusions by resin plug (RP) and the reduction of tubular diameter by the presence of crystal-like filler particles. ED, exposed dentin; T, tubule; RP, resin plug; DS, dentin sub-surface.

dissolving (Figure 5E), but the tubular apertures (Figure 5F) remained occluded.

UDS formed a smooth amorphous layer that contained particles ~ 0.5 μm in diameter, over dentine (Figure 6A). Particles had a tendency to form clusters and adhered to the underlying dentine completely occluding the tubular orifices (Figure 6B). Longitudinal sections showed the dentine surface covered by a coating of UDS that was ~ 0.4 μm thick and plug-like structures in the tubules (Figure 6C). After exposure to oral environment for 7 days (sub-group 2), the dentine surface treated with UDS showed a residual coating of dentine with different representations of crystal-like particles (Figure 6D). Longitudinal sections showed a thick granular surface and peritubular dentine masking the intratubular space (Figure 6E).

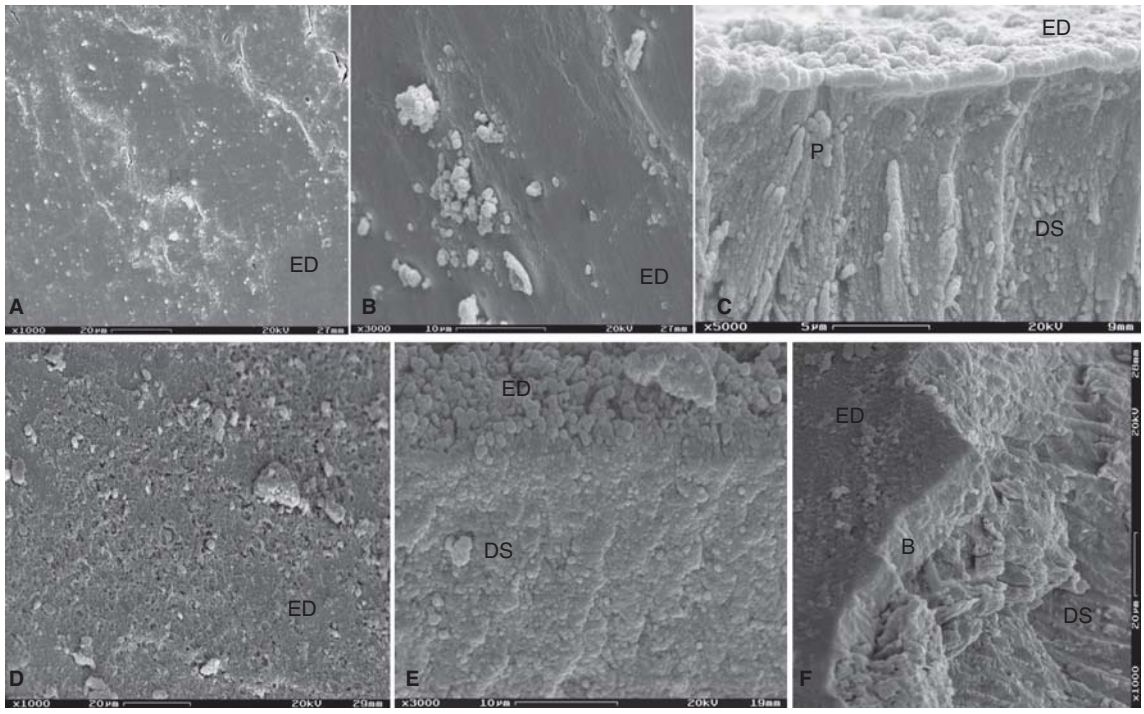

Figure 6. Representative SEM micrographs of Universal Dentin Sealant on the exposed dentin surfaces showing the smooth amorphous layer with particles (A) forming clusters (B). The thick layer of varnish completely covers the tubular orifices. Longitudinal sections of exposed dentine (C) detect the penetration of the resin sealant into dentinal tubules forming varnish-tags inside the tubule. Exposure to oral fluids for 7 days may have solubilized the varnish allowing exposure of the underling smear layer on ED (D). Transverse sections of exposed dentine show a thickening of inter-diffusion and peritubular dentine (E). Fractures between the exposed surface and the inter-diffusion reveal that inter-diffusion forms a barrier-like structure (B) with tag-like structures reproducing tubular dentine morphology after 7 days exposure. ED, exposed dentine; T, tubule; DS, dentine sub-surface.

Occasionally, small areas of separation between the surface coating and the dentine sub-surface demonstrated the presence of a barrier-like structure, with tag-like structures reproducing the tubular dentine (Figure 6F).

FOV-treated dentine surface exhibited an amorphous layer with dispersed particles leaving most of the tubules partially occluded (Figures 7A and B). Transverse sections of exposed dentine revealed a thick coating of varnish almost blocking the tubular apertures (Figure 7C). After 7 days of exposure to the oral environment (sub-group 2), ED showed areas of solubilization of a surface coating with disclosure of the underling smear layer (Figure 7D). The solubilization process involved the tubular blocks of varnish on ED simultaneously showing crystal-like precipitates with reduction of the tubular diameter (Figure 7E).

Clinical study

The mean VAS scores are shown in Table II. There was no difference among baseline VAS scores of all groups ($p > 0.05$). After treatment, all teeth exhibited statistically significant reductions in VAS in Post-1. Teeth treated with VF had lower VAS scores immediately after Post-1 control (VF vs FOV: $p = 0.034$). After 7 days of exposure to oral fluids (POST-2) there was no significant difference among tested materials, according to Sidak's multiple testing adjustment. However, when compared with baseline data, all the VAS scores at post-treatment evaluation points were significantly decreased ($p < 0.05$).

Discussion

Extensive tubular occlusion and permeability reduction reported for various classes of materials when treating DH reflect intrinsic material performance, but they show differences in terms of experimental design and execution [22]. As suggested by Gillam et al. [40], *in vitro* evaluation of desensitizing agents is gathered by using human dentine discs with fluid filtration systems for hydraulic conductance measurement (i.e. dentinal permeability) [3] under simulated oral cavity conditions. SEM images are made of the morphological changes in material-treated dentine surfaces before and after exposure to oral fluids to determine the stability of tubular occlusion [5,7,8,16,18]. One advantage of these studies is that the physical and chemical influences that affect tubular occlusion (i.e. toothbrush, dietary acids and saliva) can be evaluated separately to simplify interpretation of data and within a specific time framework. However, morphological evidence of

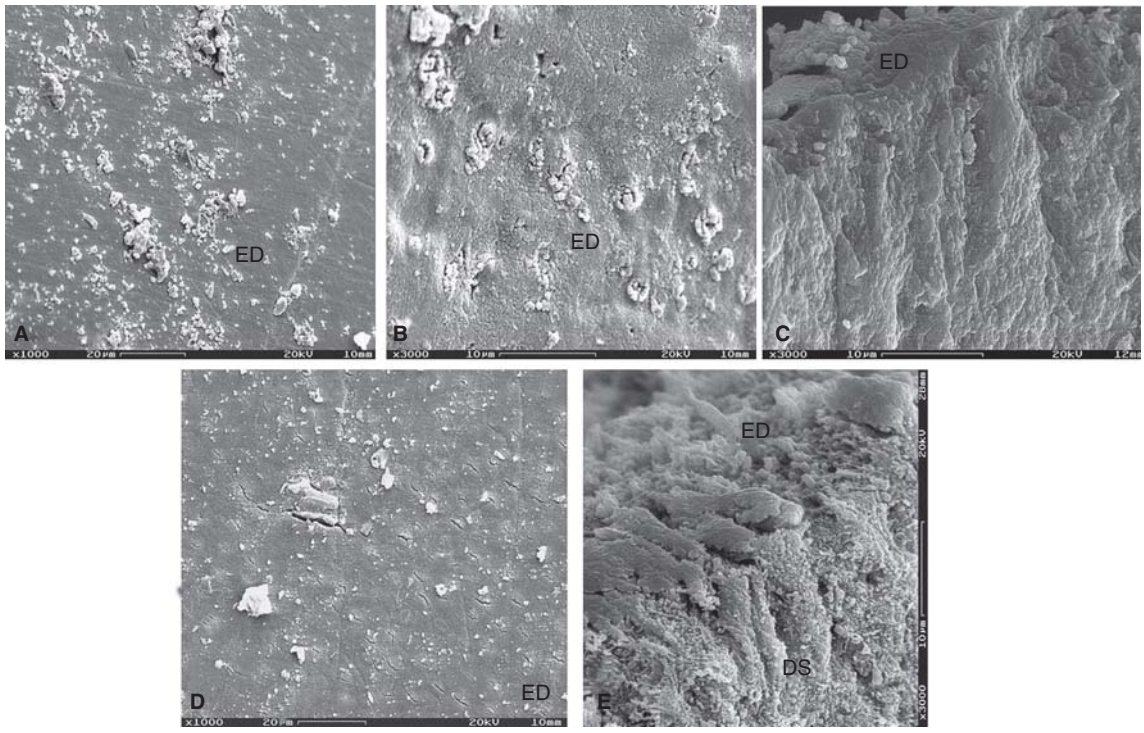

Figure 7. Representative SEM micrographs of the exposed dentine surface after application of Flor-Opal® Varnish showing a layer with dispersed particles (A and B) that partially obliterated tubule orifices. Longitudinal sections (C) showing the thick cover of varnish which blocks the tubular orifices. Exposure to oral fluids for 7 days largely solubilized the varnish leaving a surface of smear layer on ED (D). Transverse sections of exposed dentine (F) show the solubilization of the tubular blocks of varnish in ED and crystal-like precipitates just below the tubular apertures (E). ED, exposed dentine; T, tubule, DS, dentine sub-surface.

Table II. Visual Analogue Scale (VAS) values measured in 30 patients at baseline and post-treatment.

	*Vertise flow *M* (SD)	$^{\circ}$Universal Dentine Sealant *M* (SD)	†Flor-Opal Varnish *M* (SD)	Anova one-way
PRE-1	5.4 (2.2)*	5.8 (2.3)$^{\circ}$	4.7 (1.9)	NS
POST-1	0.5 (1.1)*	0.6 (0.8)$^{\circ}$	1.9 (1.5)†	NS$^{*\circ}$ 0.04*† 0.02$^{\circ\dagger}$
POST-2	1.7 (1.2)*	1.2 (1.1)$^{\circ}$	1.8 (1.5)†	NS$^{*\circ}$ 0.01*† 0.03$^{\circ\dagger}$
Anova one-way	< 0.01	< 0.01	< 0.01	

Values expressed as means and standard deviation.
*Vertise Flow; $^{\circ}$Universal Dentin Sealant; †Flor-Opal Varnish.

materials' performance in oral environment conditions as well as the longevity of the tubular occlusion under environmental stress may not be correctly predicted for the *in vivo* situation. Furthermore, prolonged exposure to environmental fluids would be essential to observe the behaviours of different materials on dentine. For instance, instability of the resins in an oral environment [9] and in simulated oral cavity conditions [33] increased within months of observation. The formation of bio-apatite by calcium-silicate cements has been observed as a gradual transformation of amorphous calcium phosphate exposed to oral fluids, within a time framework of between a few hours and up to 2 months, yielding phase mixtures richer in apatite [24,26,27].

In light of such considerations, this study aimed to assess the response of newly introduced proprietary resin-based materials as desensitizers under oral environment conditions. With this assessment we decided: (1) to conduct an ESEM-EDX examination in order to investigate the semi-quantitative elemental composition and micro-morphology of the matrices; this might reveal information regarding their mode of action [32,34,41]; (2) to investigate the SEM morphology of the material-treated dentine and the tubular occlusions after exposure to oral fluids (and environment) which might reveal the materials' behaviour on the dentine surface and their occlusion capacity [24,27,33]; and (3) to compare the morphological features with the clinical

outcomes of the resins by employing VAS measurement of pain, which might allow for correlation between form and function of the tubular seals [9,38].

The morphological and clinical studies were conducted on hypersensitive teeth as part of a study population of 286 hypersensitive teeth using the same exclusion/inclusion criteria, in order to compare data on teeth as homogeneous as possible. Even if the *in situ* SEM-replica technique was utilized to accurately trace the material-treated tooth surfaces [9], the use of extracted specimens might show information on dentine cross-sections with the interpretation of peritubular and intratubular dentine interactions of the desensitizers after exposure to oral fluids.

Furthermore, a NaF varnish was used in our investigation as a control due to the effect of fluoride on tubular occlusions [6,38] as well as in the reduction of the VAS measurement of pain [10,11,13].

Data clearly showed that environmental interaction modifies the morphological aspect of all the material-treated dentine surfaces and tubular occlusion. However, different responses could be observed as a consequence of the material composition and interaction capacity with the dentine in an oral environment.

In the control group, FOV fluorine varnish was somewhat solubilized from the ED surfaces and in the tubule occlusions as possible evidence of lack of bonding between the varnish and the dentine [10] after 7 days in the oral cavity. At the same time, crystal-like precipitates were observed in the tubules with a reduction of the tubule's radius. These observations are interpreted as a consequence of the complex series of chemical and physical interactions involving the F ions in the varnish and the Ca and P in the dentine, which produce a mechanical obstruction of the tubules by precipitation of Ca-P phases [6]. Markowitz and Pashley [14] claimed that any substance that causes a decrease of tubular radius is able to reduce clinical symptomatology of DH by reducing fluid conductance. Therefore, the presence of crystal-like precipitates inside the tubules would have produced a relief of DH. Following treatment of hypersensitive dentine with FOV, we clinically observed a decreased of the VAS measurement compared to the baseline, in POST 1, immediately after the application, and in 7 days of exposure after treatment. Compared to the baseline, the reduction in VAS was significant in both POST-1 and POST-2, but it was not in POST-2 if compared with POST-1 values. It is likely that, immediately after treatment with FOV, the tubules were occluded by both CaF_2 crystals and varnish but that, over the 7 day post-treatment time, the varnish solubilized leading tubules partially occluded with CaF_2 crystals. These results support other clinical studies on the ability of topical sealing agents, such as fluoride varnish, to reduce hypersensitivity, but whose desensitizing effects were transient, with a progressive decrease in efficacy in the post-treatment controls [10,13].

Data obtained by EDX analysis of VF self-adhering composite validate the formula reported by Wej et al. [33]. Furthermore, this investigation detected Ca and Al in the elemental composition of VF. Si, Yt, F and Ba were the main elements and would be utilized as filler components in the resin [33]. This is in accordance with different studies that reported the use of ytterbium-fluoride and barium fillers with the purpose to increase radio-opacity [42,43], shorten the setting and increase hardness in composite matrices. Fillers of ytterbium-fluoride have been associated, moreover, to the fluoride release on the media due to the leach of surface-retained fluoride [44] with mineralization effects on the tooth's surface.

Morphologically, the application of VF formed a thick coating layer with particles that were tightly adapted to the ED surface and which completely masked the tubules. However, this SEM investigation was not able to show an inter-diffusion zone of VF in the dentine, possibly due to a very thin layer of resin–dentine infiltration (i.e. 200 μm) which could not be detected at the standardized magnifications used. Clinically, VF produced a significant drop of the VAS value in POST-1 compared with the baseline, presumably because the self-adhering flowable composite produced a tubular seal [44]. Our observations are in agreement with previous studies [45] that described the intimate interface between VF and dentine using transmission electron microscopy.

The evidence of particles in the thickness of the VF layer may be explained by (i) the acidic phosphate group of the self-etching composite, which could have raised ionized Ca and P ion concentration from the dentine, to a point where it exceeded the product's solubility constants [46]; and (ii) the consequent precipitation of Ca and P on the dentine [47]. Alternatively, the particles may simply have been insoluble fillers in a light-cured polymerized matrix.

The resinous layer formed by VF on the dentine showed the ability to resist 7 days in the oral environment, supporting our hypothesis that the coating remained on the dentine surface and in dentine tubules. In fact the composition of the material reflects longevity of tubular occlusions [5,17]. Furthermore, the interaction with saliva ions and the presence of F ions in the composite might have supported the growing of crystal-like precipitates on the ED surface and in the tubules exposed to oral environment conditions [6]. Regardless of the mechanism of tubule occlusion, this work suggests the ability of VF to occlude dentinal tubules in DH treatment. On the other hand, the evidence of crack and gap formations on the ED surface may imply instability of the polymer matrix under oral conditions. We related cracks on the ED in sub-group 1 to

dehydration of the samples during the SEM procedure. Nevertheless, the presence of cracks and the formation of gaps may suggest a weakness of bonding between dentine and resin composite in an oral environment [48]. This speculation is supported by recent investigations that documented hydrolytic instability of VF in water [33]. The hydrolysis of the interface between nano-sized filler particles and polymer matrix may create diffusion paths for water. Thus, the evidence of cracks and gaps may indicate resin–filler interface degradation within 7 days of exposure to saliva and the oral environment [33].

Spectra in UDS treated teeth revealed Ca, Cl and Si as the elements in highest quantity in the matrix, which also contains Al peak and precipitates rich in Ca and Cl.

Morphologically the behaviour of the resin sealant was very different to that of the self-adhesive composite. A surface coating was clearly evidenced on the dentine under SEM. Plug-like structures of particles were also detected in the tubules. Both features may have contributed towards a significant decrease of VAS value in POST-1 compared with the baseline [14,22,23,34]. However, one of the most important outcomes of this study was that the 7 days of oral function strongly changed the morphology of UDS on the dentine, giving rise to a dense barrier-like structure with tag-like structures resembling demineralized tubular dentine. Thus, we believe that the 7 days of fluid contact and oral environment conditions would be essential for the morphological formation/expression of a dense seal into the exposed dentinal tubules.

As a result of this investigation, we observed morphological differences in the features of the seal and tubular dentine occlusion between VF and UDS, after 7 days of exposure to an oral environment. Thus, the null hypothesis that the resin-based material-treated dentine surfaces showed no morphological difference after 7 days in an oral environment was rejected.

Clinically, all the materials tested produced a reduction of dentine permeability. In addition, after 7 days, POST 2, there was no statistically significant difference in the decrease of the VAS, irrespective of the desensitizing agent employed. These considerations are in accordance with the literature, whereby significant differences among desensitizing effects may appear in longer term evaluations [10,12,13].

Further research in this field is needed to better clarify the effectiveness of FV and UDS in long-term clinical trials.

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