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LONG TERM COMPARATIVE STUDY ON THE EFFICACY OF RESIN-BASED MATERIALS IN DENTAL HYPERSENSITIVITY

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Introduzione

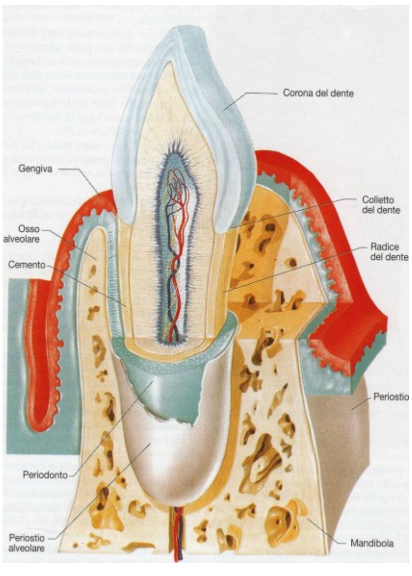
La sensibilità dentinale è stata oggetto di numerosi studi sin dal 1678 con le prime osservazioni scientifiche al microscopio ottico (Leeuwenhoek), che portarono alla luce la tipica conformazione tubulare della dentina.

All'inizio del novecento si formularono i primi meccanismi che spiegano questo fenomeno; Alfred Gysi affermava infatti che: "i tubuli dentinali sono privi di qualsiasi sostanza nervosa, solo il fluido contenuto in essi poteva, con il movimento, scatenare una risposta di tipo dolorifico percepita dalla ricca rete nervosa contenuta all'interno della polpa".

Solo dopo circa sessant'anni Martin Brännstrom, supportato da numerosi studi sperimentali, propose la sua "Teoria Idrodinamica" con la quale spiegava gli effettivi meccanismi che stanno alla base della sensibilità dentinale.

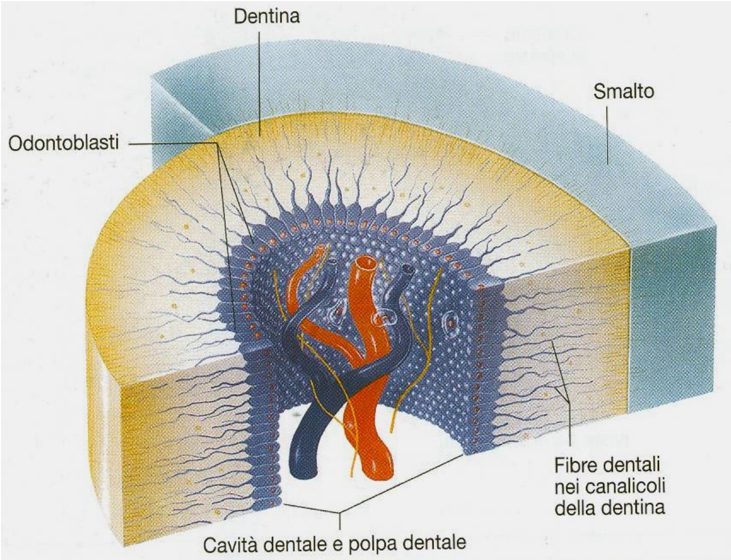
Struttura Dentinale

La nostra attenzione verrà concentrata sulla dentina e sullo strato periferico odontoblastico pulpare, oggetto dello studio.



Dentina

La dentina, chiamata anche avorio, è un tessuto connettivo mineralizzato prodotto dagli odontoblasti, si presenta come una sostanza fondamentale calcificata e possiede meno durezza dello smalto.



Il costituente principale della matrice organica è il collagene di tipo I (18%) disposto in sottili fasci di fibrille, contiene anche macromolecole non collagenosiche come idrossiapatite (70%) e acqua (12%).

Costituisce il corpo del dente e ne determina la cromaticità.

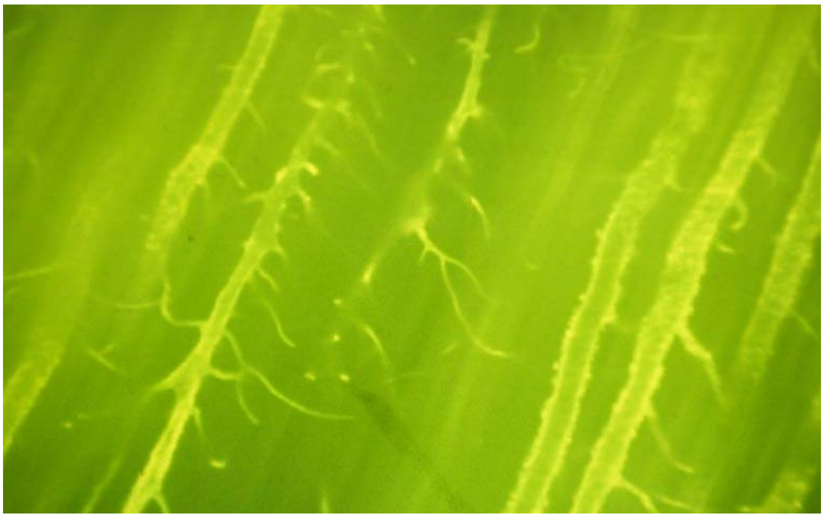
Appare come un tessuto di colore giallo sottostante lo smalto; essa ha la funzione di proteggere la polpa dalle variazioni di temperatura e dalle sollecitazioni meccaniche.

La dentina è un tessuto dinamico, cioè va incontro a modificazioni strutturali in risposta a svariate cause come l'invecchiamento, la carie o l'abrasione.

È percorsa da tubuli dentinali che si dispongono radialmente dal cavo pulpare alla giunzione amelo-dentinale, nella porzione coronale, e a quella dentina-cemento nella parte radicolare.

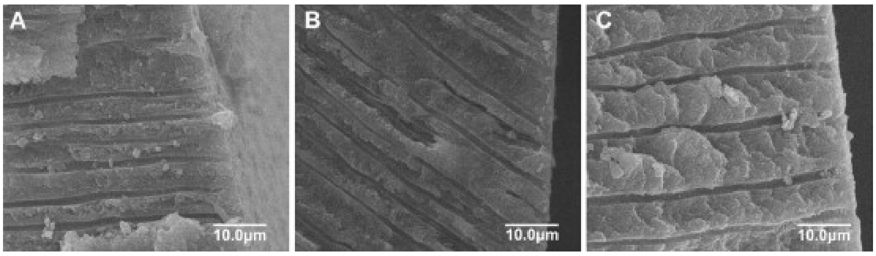
Ciascun tubulo è rivestito da un collare di dentina ipermineralizzata chiamata dentina peritubulare al cui interno scorre un fluido con una pressione di circa 20 mm di mercurio, detto fluido dentinale.

I tubuli dentinali hanno un percorso a S con frequenti anastomosi laterali, per gli scambi nutritizi, il loro diametro non è uniforme , ma varia da 2.5 micron in prossimità della polpa a 0.9 micron a livello della giunzione amelo-dentinale



Struttura tubulare della dentina radicolare osservata al microscopio confocale.

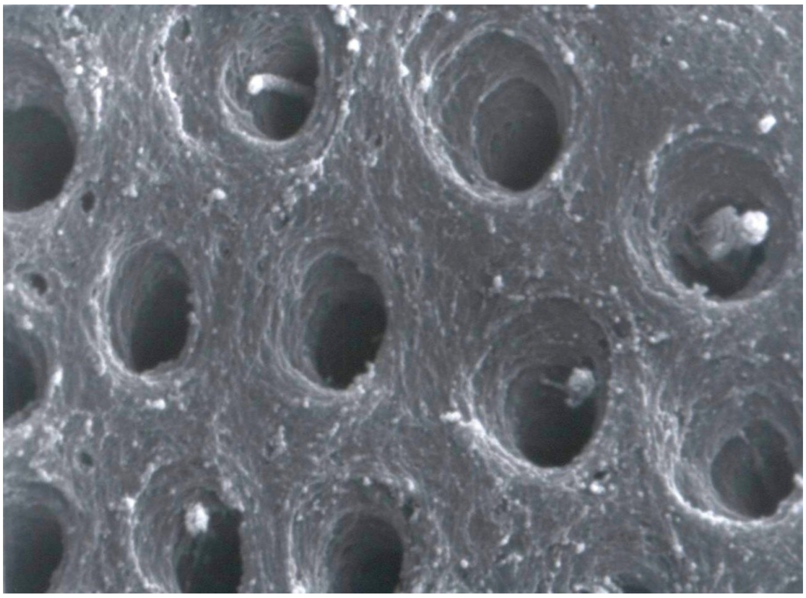
Anche il loro numero è variabile, infatti si parte da 45000/mm^2 a livello della parete pulpare, a 19000/ mm^2 a livello della superficie esterna (giunzione smalto-dentina).



Visione al SEM di tubuli dentinali in sezione longitudinale

All'interno del tubuli, oltre al già citato fluido, sono presenti anche le fibrille di Tomes, ovvero il processo citoplasmatico periferico degli odontoblasti, chiamato anche prolungamento odontoblastico.

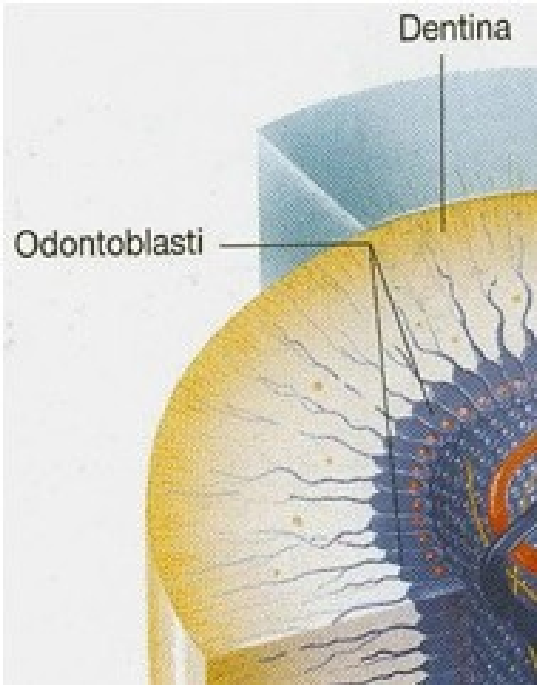
I tubuli sono separati l' uno dall'altro da dentina intercanalicolare.



Visione al SEM di tubuli dentinali in sezione trasversale

Strato odontoblastico periferico

È costituito da elementi specifici, gli odontoblasti, che giocano un ruolo fondamentale nella sintesi del collagene della matrice predentinale; questa sintesi intracellulare produce la sostanza fondamentale extracellulare e le relative fibre collagene che formano il reticolo fibroso della predentina (1); provvedono poi alla mineralizzazione della dentina.



La secrezione della matrice precede la mineralizzazione e questi due processi sono separati nel tempo e nello spazio dalla predentina.

Man mano che avviene la deposizione di dentina (dentina secondaria) gli odontoblasti si spingono in direzione centripeta, quindi verso il centro

della polpa, andando a ridurre progressivamente, con l'età, lo spazio endodontico.
Per quanto riguarda il processo odontoblastico, a tutt'oggi è ancora dibattuta la sua vera estensione, ovvero se raggiunga la giunzione amelo-dentinale.
Dopo la formazione iniziale di dentina gli odontoblasti, attraverso il prolungamento, possono ancora modificare la struttura dentinale producendo dentina peritubulare, la quale produce la diminuzione del diametro tubulare.
Se sottoposti a stimoli algogeni gli odontoblasti possono accelerare la formazione di questo tipo di dentina e collagene andando ad obliterare il tubulo stesso diminuendo così la permeabilità dentinale; esercitando una azione di difesa nei confronti della polpa sottostante.
Quando questo evento colpisce un'ampia area si parla di dentina sclerotica.

Ipersensibilità dentinale

L'ipersensibilità dentinale è una condizione caratterizzata dalla comparsa di dolore breve (fino all'allontanamento dello stimolo) ed intenso ascritto a nessuna patologia dentaria se non ad esposizione anatomica della dentina cervicale all'ambiente orale (2,3).
Abrasioni, erosioni, e fenomeni di recessioni della gengiva, conseguenti a root planning e scaling profondo, determinano la perdita dello smalto e/o del cemento che ricoprono la dentina superficiale a livello della giunzione amelo-cementizia, rendendo così il tessuto permeabile agli agenti esterni.
E fondamentale, per stimolare la sensibilità, sia la profondità sia la sede in cui viene applicato l'agente esterno; infatti, quanto più la cavità è profonda tanto più la dentina risulta permeabile a causa del maggior calibro dei tubuli e della loro quantità.

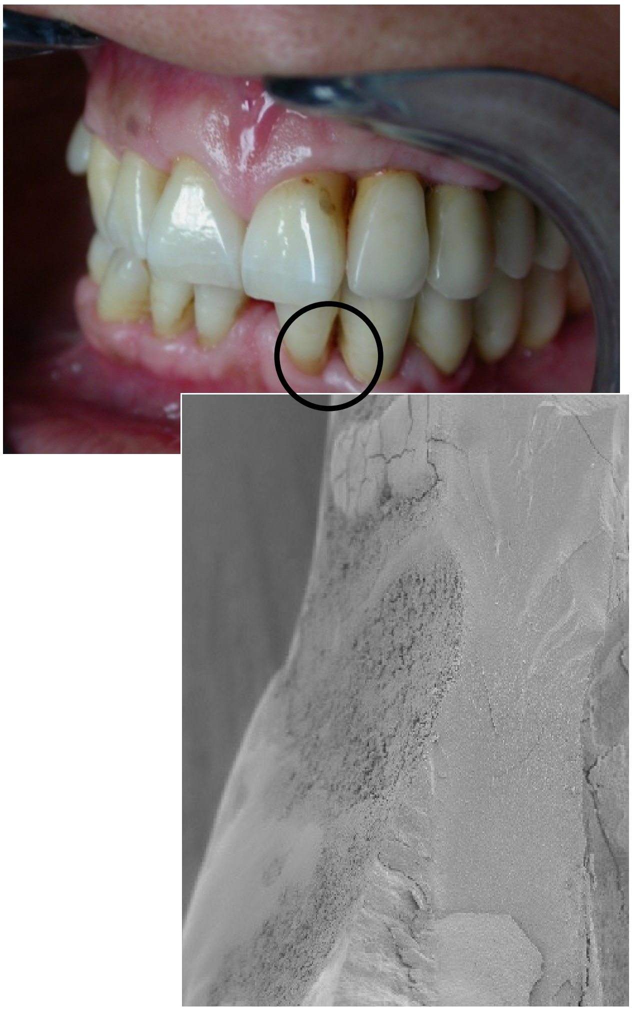
In tutti i casi, comunque, la diagnosi è difficile, soprattutto quando sono presenti processi cariosi limitrofi, nei quali le modificazioni pulpari sono simili a quelle evidenziate nei casi di ipersensibilità dentinale.

Recessioni con esposizione della dentina

Epidemiologia

La sensibilità dentinale è un'affezione di frequente riscontro nella pratica clinica, dal 4 al 74% nella popolazione adulta (4); può colpire indipendente dal sesso tutte le età, ma la massima incidenza è all'incirca tra i 35 e 45 anni, poi tende a diminuire anche se, paradossalmente, l'esposizione superficiale della dentina esposta tende ad aumentare con l'età; questo evento può essere imputabile sia alla sclerosi della dentinale, che riduce la permeabilità della dentina, che alla riduzione della sensibilità pulpare.

La sede più colpita è il colletto dei denti .



Aspetto clinico abrasione cervicale

Eziologia e fattori di rischio

Riconosciamo due cause scatenanti l'ipersensibilità:

1. L'esposizione dentinale, che provoca movimento del fluido all'interno dei tubuli con conseguente eccitazione delle fibre nervose mieliniche.
2. ipereccitabilità delle fibre nervose mieliniche della polpa.

Questi due aspetti possono essere considerati espressioni del medesimo concetto.

Fattori di rischio:

- tecniche scorrette di spazzolamento

- uso scorretto del filo interdentale
- uso di dentifrici ad elevato indice di abrasività
- presenza di sostanze acide di origine esogena o endogena nel cavo orale
- traumi cronici dovuti ad abitudini viziate (digrignamento dei denti in situazioni di stress)
- piccole fratture dentali
- perdita di sostanza dentale al colletto del dente presumibilmente dovute a trauma occlusale (abfraction)
- carie a livello cervicale (5° classi di Black)
- interventi di chirurgia parodontale
- esposizione di dentina al colletto associata a perdita di gengiva aderente
- difetti dello sviluppo di smalto e dentina
- otturazioni fratturate
- preparazioni protesiche (esposizione di 70000 tubuli per mm2).

Sulla base di queste osservazioni, oggi, sono stati preparati soluzioni desensibilizzanti in grado di agire a due livelli:

1. Occlusione dei tubuli dentinali, con conseguente riduzione della permeabilità dentinale.
2. Riduzione dell'attività delle fibre nervose della dentina, e quindi della trasmissione dello stimolo algico ai centri nervosi superiori.

Sintomi e aspetti clinici

L'ipersensibilità dentinale è caratterizzata da un dolore transitorio in risposta a stimoli esterni di tipo meccanico, termico, chimico, evaporativo o chemio-osmotico che si verifica in denti con dentina esposta senza l'evidenza di altri effetti o patologie.

La sensibilità dentinale può colpire tutti i denti, ma prevalentemente colpisce canini e primi premolari quasi esclusivamente a livello delle superfici vestibolari.

La dentina sensibile può presentarsi anche su altre superfici, includendo cuspidi e angoli incisali, e sulle superfici orali (questo è spesso in correlazione con reflusso gastro-esofageo).

È vero anche che non tutte le superfici dentinali esposte evocano dolore e non tutte le regioni della dentina sensibile sono uguali: infatti esse variano per estensione e sensibilità a stimoli differenti.

Ad esempio è osservato come i denti ipersensibili rispondono ad uno stimolo come il freddo e non al tatto.

Fisiopatologia del dolore e fisiologia della polpa e della dentina

La polpa è riccamente innervata ed è costituita da fibre nervose sia di tipo A (mieliniche) sia di tipo C (a mieliniche). Queste comprendono sia fibre sensitive afferenti, responsabili della trasmissione dolorifica,
sia fibre simpatiche efferenti, coinvolte nella modulazione del microcircolo pulpare.

Le fibre nervose afferenti sensitive che arrivano al dente sono composte da neuroni bipolari, il cui corpo cellulare è localizzato nel ganglio di Gasser, queste fibre fanno capo al nucleo sensitivo principale e al nucleo della radice spinale del trigemino, mentre i neuroni periferici decorrono con due delle branche trigeminali, la mascellare e la mandibolare.

Sia le fibre mieliniche sia le amieliniche sono provviste di caratteristiche fisiologiche differenti: le fibre A sono in grado di condurre gli stimoli più rapidamente delle fibre C.

Il dolore evocato dalla stimolazione delle fibre A è qualitativamente diverso da quello provocato dalla stimolazione delle fibre C; infatti il primo è acuto, penetrante o pugnalante, mentre il secondo sordo, bruciante e di difficile sopportazione.

In caso di preparazione cavitaria conservativa, quelle che rispondono maggiormente sono le fibre A mentre quelle C sono interessate da stimoli potenzialmente capaci di danneggiare i tessuti.

Secondo gli studi di Närhi, sembra che le fibre A siano responsabili della sensibilità dentale, mentre le fibre C rispondono solo quando gli insulti esterni raggiungono la polpa (sensibilità pulpare).

La maggior parte delle fibre nervose entra nel dente attraverso il forame apicale, si raggruppa in fasci paralleli al centro della polpa e insieme al connettivo va a formare un fascio neuro vascolare; inoltre, mentre il gruppo più consistente di questi è situato nella regione centrale pulpare, fibre sporadiche sono diffuse alla periferia.

I nervi attraversano la polpa radicolare senza emettere collaterali e gli assoni non si diramano fino a quando non raggiungono la polpa coronale, ove si aprono a ventaglio suddividendosi in piccoli fasci nervosi.

La rete neurale così formata andrà a costituire il plesso sottodontoblastico o di Raschkow, dal quale le fibre si estendono verso la dentina, alcune di queste tornano indietro verso la polpa coronale, mentre altre terminazioni nervose attraversano lo strato odontoblastico.

Le suddette terminazioni presentano delle dilatazioni finali che hanno un aspetto caratteristico detto a collana e grazie a Gunji e agli studi sui nervi terminali, oggi possiamo classificare, nel plesso sottodontoblastico, quattro tipi di terminazioni nervose:

- fibre marginali che non raggiungono la predentina e terminano negli spazi extracellulari dello strato odontoblastico;
- fibre predentinali, che si estendono sino al limite odontoblasti-predentina o impegnano quest'ultima;
- fibre predentinali, che si ramificano copiosamente nella predentina;

• fibre dentinali, che passano la predentina senza emettere collaterali e penetrano la dentina attraverso i tubuli, con un calibro con diametro decrescente (da 4 a 1 millimicron) dall'interno all'esterno.

Queste ultime entrano nei tubuli e giacciono in stretta associazione con i processi odontoblastici.

Lo strato di penetrazione nella dentina delle fibre è molto vario (da pochi micron sino a 100 millimicron) ma, sostanzialmente, possiamo dire che la dentina è in gran parte sprovvista di fibre nervose. Tradizionalmente, gli studi sull'innervazione della dentina sono stati condotti tramite impregnazione argentica; sfortunatamente, i risultati sono stati in parte falsati dal fatto che anche altre strutture, come le fibre reticolari e collagene, assumevano questa colorazione.

In seguito, tale difficoltà è stata superata mediante la digestione enzimatica del collagene. Studi più recenti, grazie all'ausilio della microscopia elettronica, hanno dimostrato come sia difficile differenziare un piccolo terminale nervoso non mielinizzato da un corpo o processo cellulare odontoblastico.

D' altra parte, Frank ha dimostrato che nella predentina e nella dentina interna le fibre nervose presentano un decorso rettilineo lungo i processi odontoblastici, con i quali mantengono stretti rapporti.

Questi ultimi, si presuppone, potrebbero funzionare da trasduttori, mantenendo un rapporto tra la dentina esterna e quella interna; è quindi possibile ipotizzare la presenza di forze elettriche a bassa resistenza tra odontoblasti e terminazioni nervose.

Teoria Idrodinamica

Brännstrom (2) ha ipotizzato che il fluido dentinale sia sottoposto alle stesse leggi della fisica, che regolano i movimenti dei liquidi nei capillari vetrosi.

Questo movimento provoca una deformazione dell'odontoblasta o del suo processo, e di qui i meccanorecettori simili della polpa trasmettono il dolore dato dal rapido movimento del liquido verso le fibre nervose della polpa. Tale processo, detto di aspirazione cellulare, è frequente in quelle otturazioni non dovutamente sigillate o nella dentina esposta, ma non è di certa correlazione con lo stimolo doloroso, come prova. Sotto stimoli, quali getti d'aria, si causa un movimento del fluido tubulare verso la polpa e le sue fibre nervose, causando lo stimolo doloroso.

Ne consegue che il paziente riferisce sintomatologia dolorosa agli stimoli caldi, freddi, elettrici, chimici, disidratanti e osmotici. Infatti, il contatto con sostanze fredde provoca una contrazione del fluido, mentre lo stimolo caldo produce un movimento centripeto verso l'estremità pulpo-dentinale.

Il dolore suscitato da una prolungata applicazione di uno stimolo caldo è di tipo sordo, completamente diverso da quello provocato dal freddo che è acuto (2).

Quindi, la letteratura moderna suggerisce che il dolore evocato può essere di due tipi, a seconda che il movimento del fluido dentale sia centripeto o centrifugo; a conferma abbiamo che stimoli caldi richiedono un maggiore intervallo di tempo nel manifestarsi rispetto a quello scatenato da stimoli freddi (5).

Si è concluso, pertanto, che un rapido reflusso nel versante pulpare dei tubuli dentinali può determinare lo stiramento sia dei processi cellulari sia delle fibre nervose nei tubuli stessi e nella polpa limitrofa.

Permeabilità dentinale

L'incremento della sensibilità dentinale può essere causato sia da un aumento del flusso centrifugo del liquido attraverso la dentina che da un aumento dell'eccitabilità nervosa.

Accettando la teoria idrodinamica possiamo affermare che il fluido dentinale, responsabile della trasduzione di una varietà di stimoli fisici in attività nervosa, per attivazione delle fibre A, può essere quantificato misurando la conduttanza idraulica della dentina (Lp).

La conduttanza è condizionata, secondo la legge di Hagen-Poiseuille, da numerose variabili quali: la pressione del fluido che si muove attraverso la dentina, la lunghezza dei tubuli dentinali, quindi lo spessore dentinale; la viscosità del fluido stesso e il raggio dei tubuli (6).

Il fattore più importante è la quantità di tubuli presenti, ma ancora di più lo è il diametro degli stessi, in quanto la conduttanza è proporzionale alla quarta potenza del raggio, quindi piccole variazioni di raggio possono avere grandi effetti sul flusso del fluido (7).

Anche la dentina di reazione ha effetto riduttivo sulla permeabilità, infatti questa dentina è costituita morfologicamente da un numero inferiore di tubuli orientati disordinatamente verso la polpa.

Anche lo smear layer e lo smear plug possono condizionare la conduttanza idraulica dentinale e quindi la sensibilità.

Lo smear plug costituisce il fango dentinale che oblitera in profondità il tubulo; lo smear layer, invece, provvede allo strato superficiale esterno.

La fuoriuscita di fluido dentinale, comunque, è sempre presente a livello della dentina esposta e permeabile anche se l'entità di questo movimento non può essere sufficiente ad attivare i meccanocettori pulpari.

Qualsiasi causa che incrementi il gradiente di pressione può aumentare il movimento verso l'esterno del fluido tale da causare dolore.

Un errato spazzolamento dei denti, non solo può provocare l'esposizione della dentina permeabile ma, allo stesso tempo, riduce la lunghezza dei tubuli, aumentandone quindi il raggio e di conseguenza la permeabilità.

Recenti studi hanno messo in luce il fatto che non solo il movimento centrifugo del fluido è in grado di evocare la sensazione dolorifica, ma anche il movimento centripeto è in grado di attivare i nervi interdentali richiedendo, tuttavia, velocità ben superiori.

Terapia

L'atteggiamento terapeutico nei confronti della sensibilità dentinale si orienta in due vie:

1. La riduzione della capacità dei nervi intrapulpari a rispondere al movimento dei fluidi (8, 9);
2. La riduzione del movimento dei fluidi agli stimoli mediante chiusura dei tubuli dentinali (10).

Trattamenti domiciliari

I trattamenti domiciliari includono l'utilizzo di varie paste dentifrice agiscono attraverso entrambi i meccanismi; lo spazzolamento quotidiano con un dentifricio desensibilizzante può ridurre l'apertura dei tubuli con formazione di smear layer e plugs sull'orifizio tubulare; altre paste, quali quelle a base di potassio, agiscono innalzando la soglia del dolore dei nervi pulpari (11).

I dentifrici contenenti sostanze abrasive, come carbonato di calcio, alluminio, fosfati e silicati, occludono i tubuli dentinali direttamente, attraverso cristallizzazioni negli orifizi tubulari, o indirettamente, attraverso la formazione di smear layer e smear plug; tuttavia, lo smear layer terapeutico determina riduzione transitoria della conduttanza tubulare poichè il film sulla superfice della dentina tende a solubilizzarsi nel fluido salivare e a contatto con gli acidi alimentari.

In vitro, sono stati dimostrati

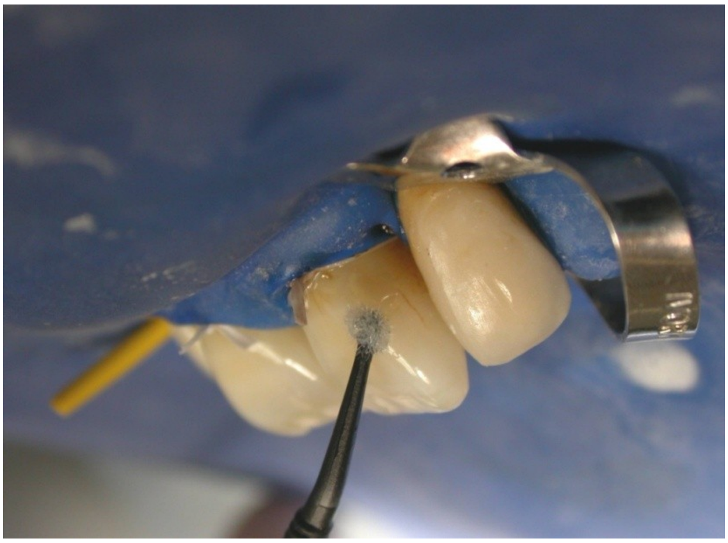
plugs resistenti all’interreazione della saliva artificiale e di acidi con l’uso di dentifrici addizionati con i nuovi materiali vetrosi bioattivi (12).

Trattamenti professionali

Nel trattamento professionale la chiusura dei tubuli dentinali è generalmente ottenuta mediante applicazione di vernici, resine e bonding agents.

Fluoro, nitrato di potassio, ossalato e calcio-fosfato sono le sostanze maggiormente utilizzate e generalmente danno luogo a cristallizzazioni endotubulari con conseguente riduzione del movimento dei fluidi (13).

Le sostanze a base di ossalato, che formano cristalli di calcio insolubili sulla superficie e plugs endotubulari profondi hanno utilizzo limitato per possibili effetti tossici (14); si tratta solitamente di soluzioni acidiche che a contatto con la superficie dentinale liberano ioni calcio che reagiscono formando cristalli di calcio.



Nella pratica clinica, vengono usati anche i bonding agents ma l’esposizione all’ambiente orale, a diretto contatto con i fluidi, favorisce la solubilizzazione del legame adesivo con la dentina (15, 16). Tutti i materiali fino ad ora utilizzati nella terapia della DH hanno dimostrato di innalzare la soglia del dolore ma limitatamente nel tempo in quanto hanno incapacità a resistere agli stress dell’ambiente orale e vanno incontro ad una progressiva solubilizzazione che ripristina i valori di iniziale sensibilità (17).

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Paper 1

Short-term response of three resin-based materials as desensitizing agents.

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Running head: Response of two resins as desensitizers

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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. ***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 analyzed 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 days 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 (1968) [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, 5, 6, 7, 8, 9] or *in vivo* Visual Analogue Scale (VAS) measurement of pain [10, 11, 12, 13, 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, 16, 17, 18, 19] and organic biomaterials or resin-based materials [20, 21, 22, 23].

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, 25, 26, 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, 30, 31, 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 behaviors 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, USA), a self-adhering resin composite, that is suggested for DH treatment, and Universal Dentine Sealant (UDS) (Ultradent, South Jordan, UT, USA) 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 prepolymerized 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, USA), 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, 36, 37].

(Table 1 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 (200X, 1000X).

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 % and atomic %) 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 program of 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 program 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 hypersensitive teeth. Patients had 30 hypersensitivity teeth (11 premolars, 13 incisors, 6 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 1. As recommended, a halogen curing light (Optilux 501, Kerr Corporation, USA; 11mm exit window) under the standard curing mode (output wavelength range: 400–505 nm; output irradiance: 580–700mW/cm2) was used to allow light curing of VF. After the treatment, teeth were immediately extracted (*N*=5 per subgroup), subgroup 1, and after 7 days post-treatment (*N*=5 per subgroup),

subgroup 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, USA) and then fractured into two halves in order to analyze 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×, 5000X).

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.

Teeth were randomly assigned to three groups (*N*=30 per group) for the treatment with the three desensitizing agents (Table 1). 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 millimeters, 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 to 60 psi, 1cm 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 seconds 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 to 40 µm particles (Fig 2). Spectra of silicion (Si), ytterbium (Yb) alumina (Al) were highest in the layer in which also phosphorus (P), calcium (Ca), barium (Ba) and fluoride (F) were found (Fig 2A).

UDS treatment left fine, dispersed particles of about 0.5 µm in a thin and smooth layer (Fig. 3). Spots on these particles showed very high pecks of Ca and chlorine (Cl) (Fig 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) (Fig. 3B).

FOV treated samples showed a layer of particles embedded in a smooth matrix (Fig 4) rich in sodium (Na) and F peaks and with traces of Si and P (Fig 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 (Fig 5A, B). Cracks were also noted in ED. Longitudinal sections showed a coating about 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 (Fig 5C). After 7 days of exposure to the oral environment (subgroup 2), tubular orifices were still not visible on ED treated dentin surface which showed cracks and gap formations (Fig 5D). Crystal-like precipitates were dissolving (Fig 5E) but the tubular apertures (Fig 5F) remained occluded.

UDS formed a smooth amorphous layer that contained particles about 0.5 µm in diameter, over dentine (Fig 6A). Particles had a tendency to form clusters and adhered to the underlying dentine completely occluding the tubular orifices (Fig 6B). Longitudinal sections showed the dentine surface covered by a coating of UDS that

was about 0.4 µm thick, and plug-like structures in the tubules (Fig. 6C). After exposure to oral environment for 7 days (subgroup 2), the dentine surface treated with UDS showed a residual coating of dentine with different representations of crystal-like particles (Fig. 6D). Longitudinal sections showed a thick granular surface and peritubular dentine masking the intratubular space (Fig. 6E). Occasionally, small areas of separation between the surface coating and the dentine subsurface demonstrated the presence of a barrier-like structure with tag-like structures reproducing the tubular dentine (Fig 6F).
FOV treated dentine surface exhibited an amorphous layer with dispersed particles leaving most of the tubules partially occluded (Fig. 7A, B). Transverse sections of exposed dentine revealed a thick coating of varnish almost blocking the tubular apertures (Fig 7C). After 7 days of exposure to the oral environment (subgroup 2), ED showed areas of solubilization of a surface coating with disclosure of the underling smear layer (Fig 7D). The solubilization process involved the tubular blocks of varnish on ED simultaneously showing crystal-like precipitates with reduction of the tubular diameter (Fig 7E).

Clinical Study

The mean VAS scores are shown in Table 2.
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 ($P < 0.05$).

Change of VAS scores over time for each desensitizing agents is presented graphically in Fig 8.

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 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 behaviors 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 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' behavior on the dentine surface and their occlusion capacity [24, 27, 33]; 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 etching 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 interdiffusion 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 etching 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 condition. 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 peack and precipitates rich in Ca and Cl.

Morphologically the behavior 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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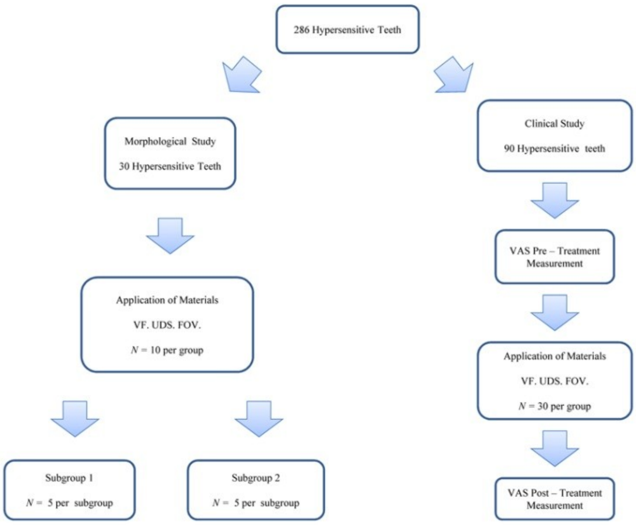


Fig 1 – Summary of the experimental design to collect hypersensitivity teeth and test different desensitising materials for the SEM morphological study and the clinical study.

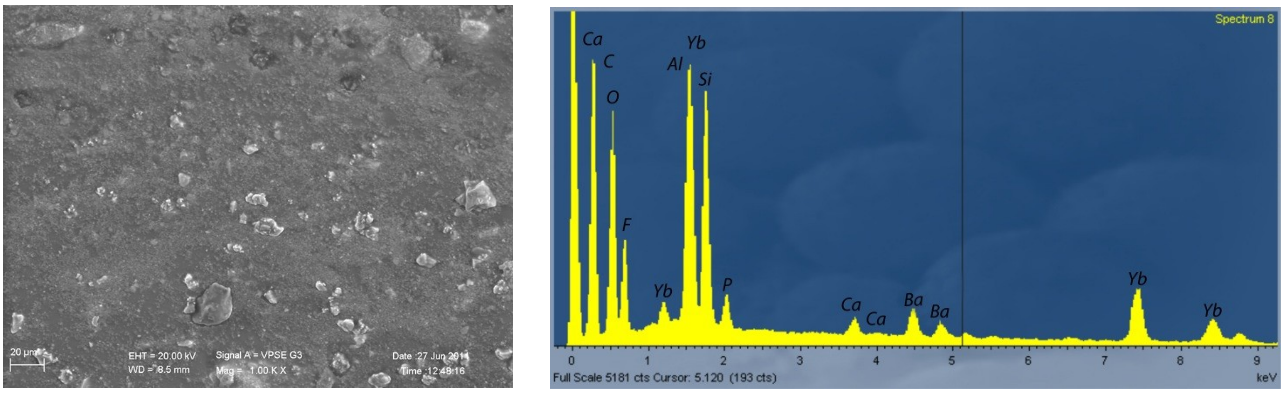
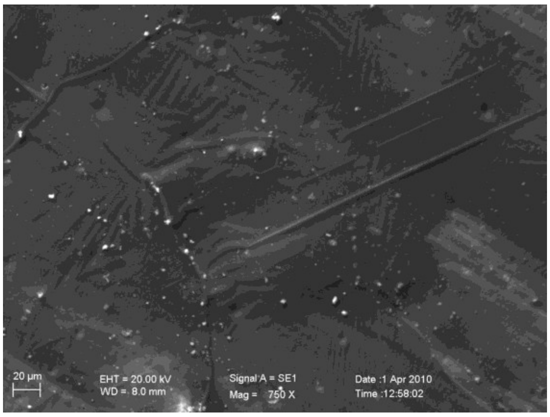


Fig 2 EDX analysis of Vertise™ Flow self etching composite showing in (A) the ESEM morphological aspect of the self etching composite composed by an amorphous matrix of nano-particles and highly visible randomly distributed 5- to 40 µm particles. EDX analysis in (B) reveals Si, Yb and F as the most represented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



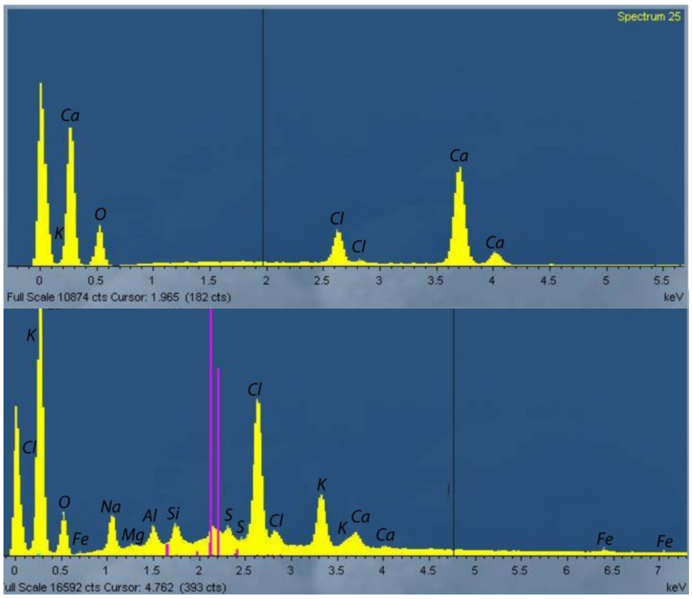


Fig 3 EDX analysis of Universal Dentin Sealant in (A) shows the ESEM morphology of the sealant composed by a smooth matrix with dispersed particles of about 0.5 µm. In (B) EDX composition of the particles with very high peaks of Ca and Cl and in (C) the semi-quantitative analysis obtained by scanning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

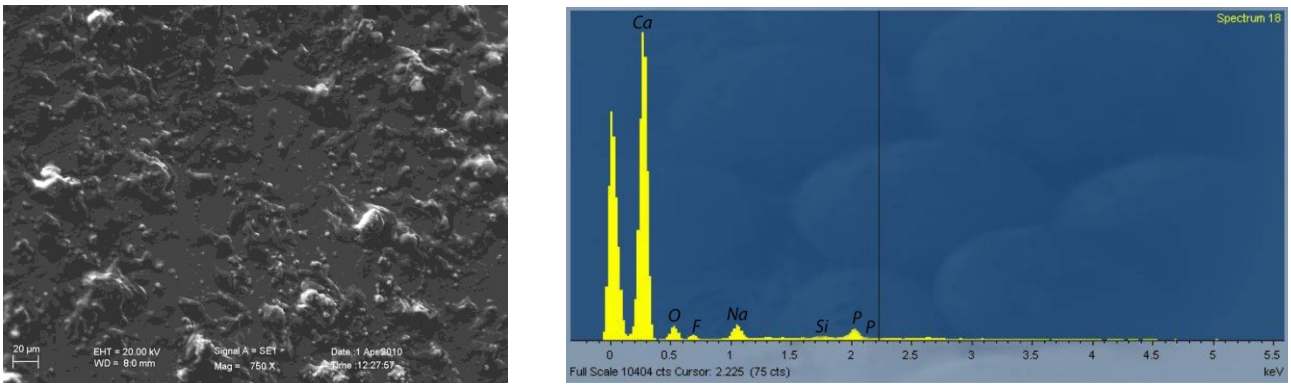


Fig 4 EDX analysis of Flor-Opal® Varnish shows in (A) the ESEM morphology of amorphous layer with particles and in (B) the semi-quantitative analysis identifying Na and F as the main elemental components. Si and P are also retrieved in traces in the varnish.

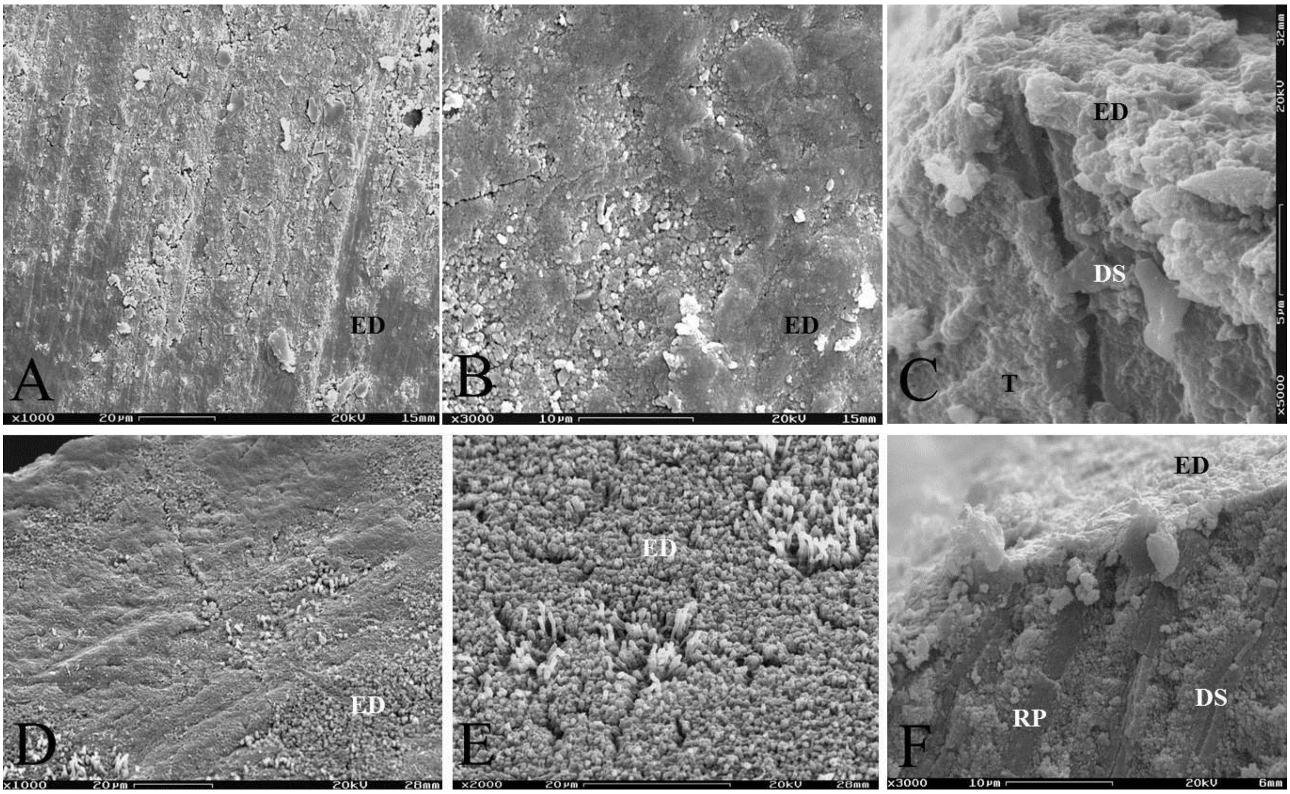


Fig 5 Representative SEM micrographs of Vertise™ Flow self etching composite immediately after application on the dentine (A, B) and after environmental exposure (D, E). In (A and B) two images at different magnifications of the dense, irregular layer containing particles masking the tubular dentine 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 etching resin composite in the dentine cannot be

disclosed by SEM under the standardised magnifications used in this study. Environmental exposure in (D and E) shows the cracks, which, compared with preaged 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 subsurface.

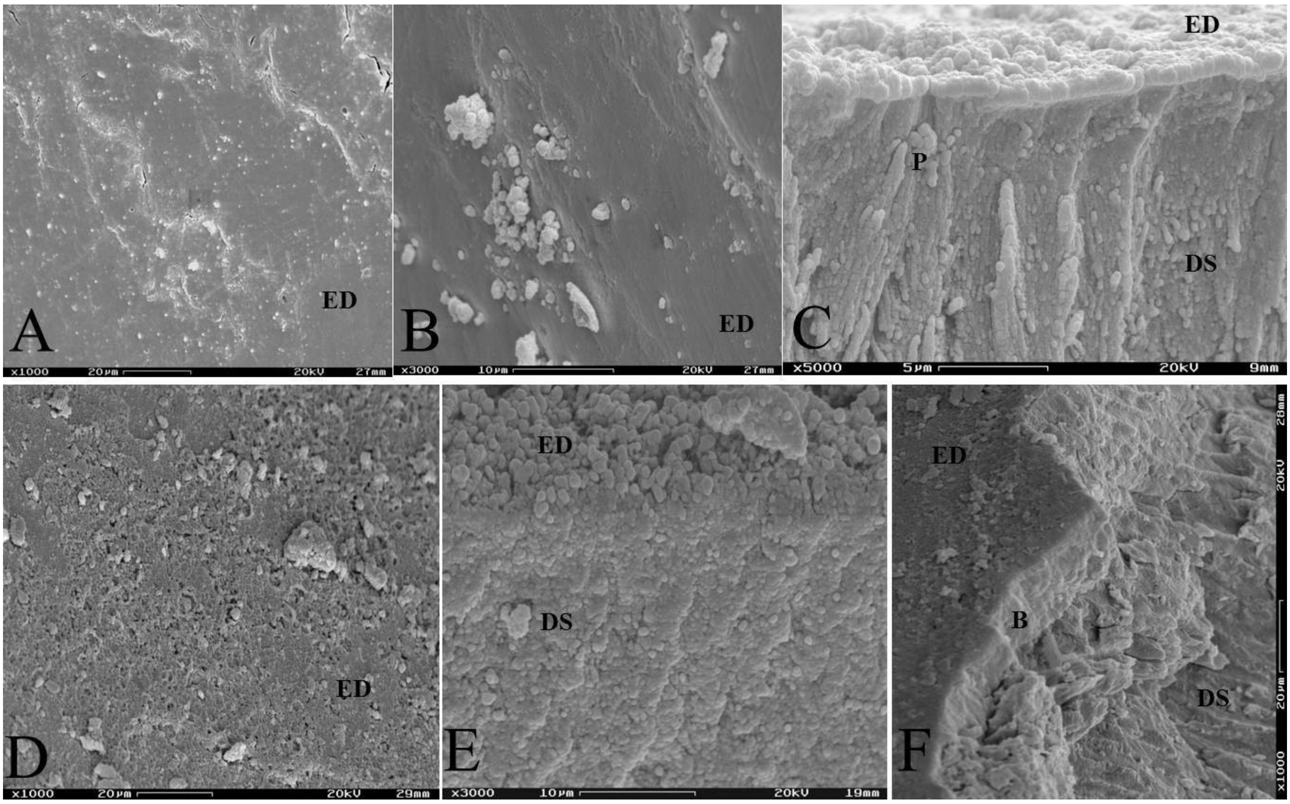


Fig 6 Representative SEM micrographs of Universal Dentin Sealant on the exposed dentin surfaces showing the smooth amorphous layer with particles (A) forming clusters (B). The thick layer of varnish completely covers the tubular orifices. Longitudinal sections of exposed dentine (C) detect the penetration of the resin sealant into dentinal tubules forming varnish-tags inside the tubule. Exposure to oral fluids for 7 days may have solubilised the varnish allowing exposure of the underling smear layer on ED (D). Transverse sections

of exposed dentine show a thickening of interdiffusion and peritubular dentine (E). Fractures between the exposed surface and the interdiffusion reveal that interdiffusion 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 subsurface.

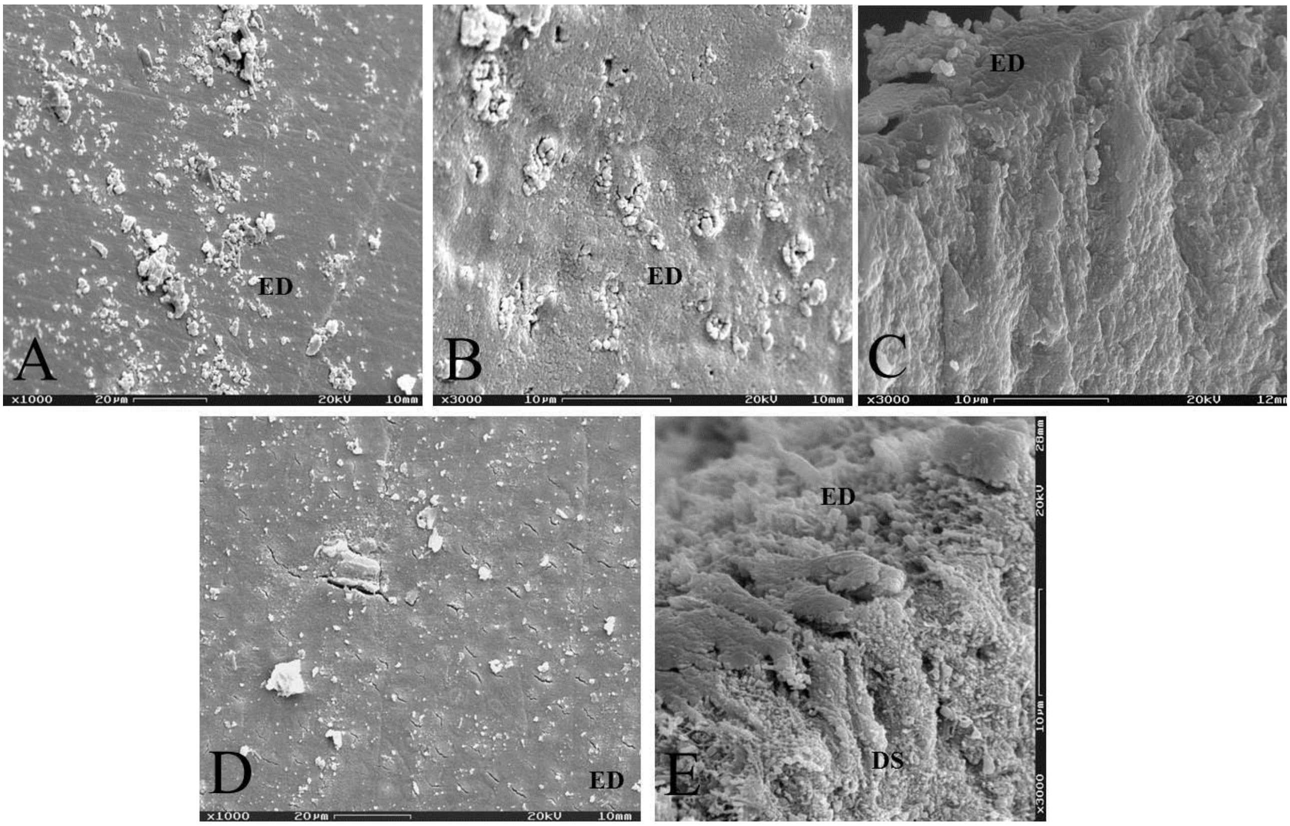


Fig 7 Representative SEM micrographs of the exposed dentine surface after application of Flor-Opal® Varnish showing a layer with dispersed particles (A and B) that partially obliterated tubule orifices. Longitudinal sections (C) showing the thick cover of varnish which blocks the tubular orifices. Exposure to oral fluids for 7 days largely solubilised the varnish leaving a surface of smear layer on ED (D). Transverse

sections of exposed dentine (F) show the solubilisation of the tubular blocks of varnish in ED and crystal-like precipitates just below the tubular apertures (E).

Table 1 Desensitising agents used in the study (manufacturer's data)

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

	GPDM (glycerol phosphate dimethacrylate) *(33)

Table 2. Visual Analogue Scale (VAS) values measured in 30 patients baseline and post-treatment. Values expressed as means and standard deviation.

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

Aim

Data emerged by the evaluation of VF after 7 days of oral environmental exposure, showed questionable interpretation about the long term clinical appliance. Questions were regarding the capacity of sealing in root hypersensitive teeth as VF/dentine surface demonstrated cracked and gaps formation after the 7 days exposure suggesting possible breakdown in efficiency when used as desensitizing agent.

With the intent to better understand the VF behavior in oral environment we decided to evaluate its efficacy as desensitizing agent by employing the VAS measurement of pain within 12 week controls in comparison to three different resin based materials.

Paper 2

Long term comparative study on the efficacy of one self-adhesive composite in dental hypersensitivity.

Bortone A, Milia E

Abstract

This paper focuses on the 12 weeks clinical effectiveness of VertiseFlowTM, (VF)a proprietary self-adhering flowable resin composite, in comparison to other resin-based materialsalso suggested as hypersensitive agentsand on an analytical study as a means to elucidate the mechanism of action. Material and Methods The elemental composition of VF, Universal Dentine Sealant (UDS),a desensitize sealant, Clearfil Protect Bond (CPB), a self-etching adhesive, and Flor-Opal® Varnish (FOV),a fluoride-varnish,were investigated by using an X-ray energy dispersive spectrometer (EDX)in conjunction with a scanning electron microscope (SEM). Data was analysed by Anova test to obtain the percent of elemental componentsof each materials. Pain reduction in hypersensitive teethwas evaluated according to the Visual Analogue Scale (VAS),before the application of the materials,immediately after,and in 12 weeks of post-treatment. Clinical data was analysed with the Student's t test for paired data, with a 5% significance level. Results an amorphous layer with particle dispersed, which composition reflects silicon, ytterbium, alumina, phosphorus, calcium, barium, and fluoride formed the matrix of VF. Clinically, all the materials were able to reduce VAS, however, at 12 weeks VF and UDS showed the higher reduction followed by PB and FOV.

With the limit of this study, data are attributed to the capacity of VF to produce effective tubular seal in dental hypersensitivity in a consequence of its proper chemical components.

Introduction

Dentine hypersensitivity (DH) is a common and painful syndrome, predominately located on the cervical part of the tooth buccal surface. DH has a wide prevalence rate (4-74%) in the adult population, with a peak in 20–50 yy [1].

DH is characterised by a short and sharp sensation of pain arising from the tubular dentine exposure as a result of enamel loss and/or gingival root surface exposure due to attrition, abrasion, erosion, abfraction or gingival recession [2]. Any thermal, osmotic and mechanical stimuli induced by the application of tooth brushing, sweet and acid foods, hot or cold drinks may provoke pain referred to fluid shifts in the exposed dentinal tubules with activation of the pulp nerves [2,3]. This phenomenon was first described in the early 20th century and later largely investigated by Brännström [2] Brännström et al 1968) becoming known as “Brännström’s hydrodynamic theory.

Most desensitizing agents have been designed to involve the dentine surface and occlude exposed tubules or penetrate the tubules, coating and sealing them.

Independently by the material used, data demonstrate a decrease of sensitivity immediately after the application of materials in respect to the pre-treatment. However, the reported clinical outcome is quite variable in long term [4-7]. Data has been explained in the capacity of the material-dentine exposed surface to resist in face to interactions with saliva and oral ambient interferences. Moreover, differences in the efficacy were attributed to the different chemistries of the materials [8-11] (7, 9, 23,31) and application modalities required by the desensitizer itself.

Several different formulations of resin-based materials have being used in DH treatment. Four different kind can be summarized:. 1) varnishes, usually with fluoride, creating a coat of calcium fluoride precipitates on the exposed surface and dentinal tubules [12,13]; 2) adhesive monomeric systems, with or without the etching phase, able to seal the exposed surface by a layer of interdiffusion in dentine and tubular resin plugs [8-10], 3) resin sealants and 4) flowable resin composites able to form covers on the dentine surface [14] which sealing capacity in the time is influenced by the resin composition and the coupling between filler and matrix [15].

Recently, new self-adhering flowable resins have been developed. According to the manufacturers, these resins bond to tooth substrate due to the presence of acidic monomers thus avoiding the need of adhesives [15, 16]. Vertise Flow (VF) has been suggested by the farm (Kerr Corporation, Orange, CA, USA), in different fields of restorative dentistry among which is the DH treatment. VF is formed by a new organic matrix of glycerol phosphate dimethacrylate (GPDM), proprietary methacrylate co-monomers, and nano-filler particles of barium glass, nano-sized colloidal-silica, nano-sized ytterbium-fluoride [15]. Recently the clinical behaviour, and morphological aspect of VF when used as desensitizing agent after 7 days of oral exposure was investigated [14]. After 7 days, VF showed a thick, irregular coat on the surface of exposed dentine with crystal-like filler particles in the tubules leading to a reduction of the Visual Analogue Scale (VAS) of dolor in a selected group of patients with hypersensitivity teeth. However, the SEM evidence of cracks and gaps in VF/dentine surfaces suggested a hydrolytic instability of VF in water by the leach of ions used in the fillers and the subsequent substitution of water in the resin mass [14,15]. Stronger adhesion in dentin was noted when VF was preceded by the appliance of an adhesive system able to impede gaps and microleakage of fluids. under stress [16].

The present study was aimed to evaluate the percentage of leachable ions in VF which may interfere with its sealing efficiency in oral fluids exposure and to investigate clinically in a comparative long term trial, the effectiveness of VF seal in DH.

The null-hypothesis tested was that, there will be no statistical differences in effectiveness between VF and three control resin material groups after 12 weeks of treatment.

Materials and Methods

Materials used

Vertise Flow ,self adhering composite , was compared to:

Universal Dentin Sealant (UDS) (Ultradent, South Jordan, UT, USA), a biocompatible, non-polymerizable, high molecular weight resin sealant in alcohol solvent;

Clearfil Protect Bond (CPB), (Kuraray, Osaka Japan) a methacrilate-based resin self-etching adhesive system;

Flor-Opal® Varnish (FOV), (Ultradent, South Jordan, UT, USA), a fluoride-based varnish.

EDX Study

To investigate the weight percentage of leachable ion species in VF mass in comparison to the other resins mass materials using an X-ray energy dispersive spectrometer (EDX) (INCA-X-acta, Oxford Instruments, Tubney Woods Abingdon, Oxfordshire, UK) in conjunction with a 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.

For the semi-quantitative X-ray analysis VF, UDS, and FOV (0.5 mg) were weighed, placed on a thin layer of Perspex®slabs. For the analysis of CPB 1 drop of self etching primer was mixed with 1 drop of adhesive resin and then layering the mixture on the slabs. All the samples were mounted on aluminum stubs (Agar Scientific, Stansted, UK). Three stubs were made for each tested material and the elemental analysis (weight % and atomic %) was performed in low-vacuum conditions (20 Pa). Atomic number, absorption, and fluorescence corrections were applied during the analysis with the ZAF correction method. Each analysis was carried out by using an area scan taken at 200 X to provide an average value for the overall composition. The result for each material was based on the average of 12 analyses (4 analyses for each of the 3 stubs in the disk).

Clinical Study

The study was designed as a split-mouth randomised clinical trial. The protocol and informed consent forms were approved by the ethics committee at the University of Sassari (n° 1000/CE). Subjects who had hypersensitive teeth were selected from an on-going program of evaluating desensitising agents at the Dental Clinic of the University of Sassari, Italy.

Two examiners selected patients complaining about hypersensitivity and who had reported this to the Department of Periodontology at the Dental Clinic. The medical and dental history of the patients was collected, and sensitive teeth were differentiated from other clinical conditions which frequently interfere with DH. To participate in the study, the subjects had to have two or three teeth that were hypersensitive to the stimulation with a blast of air.

All the subjects were thoroughly informed about the study's purpose, risks, and benefits. A total of 86 patients with hypersensitive teeth were collected. 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-planing up to 6 months prior to the investigation were excluded from the study. Other exclusion criteria were professional desensitising therapy during the previous 3 months, or use of desensitising 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 program consisted of 46 patients, 27 females and 19 males who were randomly selected from the total population of 74 subjects who had hypersensitive teeth. A total of 116 teeth (52 incisors, 38 premolars, and 26 cuspidates) were included in the study. .

The level of sensitivity experienced by the patient was considered as independent of the position of the hypersensitive tooth in the oral cavity (12).Figure 1 summarizes the experimental design used for the clinical.

Treatment Procedure

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 standardised habits during the period of the study.

Teeth were randomly assigned to four groups (N=29 per group) for the treatment with the four desensitising agents (Table 1). 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 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 to 60 psi, 1cm 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 seconds 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), after 1 week (POST- 2), 4 weeks (POST- 4) and 12 weeks (POST 5) of oral environment, the same operator carried out the sensitivity test.

Statistical analysis

The data were imputed into a datasheet for the analysis. 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. The Bartlett test for equal variance was also run. All the analyses were performed using STATA 10 for Apple. $P<0.05$ was considered statistically significant.

Results

EDX analysis

VF showed silicion (Si) ytterbium (Yb) and alumina (Al) as the highest ions in the mass and also phosphorus (P),calcium (Ca), barium (Ba) and fluoride (F) ions were detected.

The semi-quantitative analysis of UDS highlighted Ca and Cl ions associated with Si and Al, iron (Fe), chrome (Cr), potassium (K), sulphur (S), magnesium (Mg), titanium (Ti) and zinc (Zn) ions.FOV samples showed sodium (Na) and F ion peaks and also traces of Si and P ions.

Clinical Study

The study population consisted of 46 patients, 27 females and 19 males. A total of 52 incisors, 38 premolars, and 26 cuspidates constituted the group of hypersensitive teeth which were included in the study . The mean VAS score and standard error before application had a range from was 3.75±1.58 in teeth treated with VF to 4.67±1.88 in teeth treated with UDS. The difference were not statistically significant (p=0.49). After application of the different materials an important decrease in the teeth sensibility; teeth treated with VF showed the most important decrease (from 3.75 to 0.86), while the less extent decrease was noted in the FOV group (from 4.00 to 2.35). Teeth treated with UDS showed the most stable results (from 4.67 at t0, 2.85 at t1 and arriving to 2.19 at t4. Regarding the within variation into the different groups during the experiments statistically significant differences were observed in each group (repeated ANOVA measurements) (table 1)

Discussion

DH is a pain sensation that may be difficult to quantify as it largely dependent by the subjective perception of the pain. In a previous study the same research group [14], the clinical response of FV as desensitizing agent was assessed in 7 days experimental study noting questionable data in long term clinical appliance. The present study was aimed to assess the long term efficacy of VF self adhering composite using the VAS measurement of pain within 12 week controls in comparison to 1) UDS resin sealant as the sealing performances of resin flow composites may be comparable to those of resin-based sealants as in vivo and in vitro studies [18-20]; 2) PB self etching adhesive system and FOV varnish to assess the VF's desensitization effect in a range of efficiency of known classes of resin materials just reported in DH treatment [9].

VAS has been accepted as a method to evaluated DH and thus, the effectiveness of desensitizing agents in pain reduction. In clinical trials, the VAS value has been assessed thought out different stimuli, among which the cold stimulation via an air dental syringe is largely accepted [5-7].. In the present study, a VAS scale measured after a cold stimulation was used to select the study population with hypersensitive teeth as well as, in the reassessment of sensitivity until the application of the agents and in the post-treatments clinical outcomes. A 12 weeks evaluation of desensitizer's efficacy was keep in consideration in the present investigation due to the fact that significant differences among desensitising effects may appear in long term evaluation [5-7].

It has been claimed that different factors are involved in the long term effectiveness of desensitizing agents several of which concern: 1) intrinsic material performance strictly related to the different formulation of the agent [10] and the active ingredient of the material applied [4,12]; 2) stability of tubular occlusions related to the composition of the blocks [12] which finally derives by the interaction of the material components with environmental fluids [12,17].

The inorganic ions fraction of VF leachable species and that of the other resins, was also quantified using EDX spectra analysis as leachable ions may affect the sealing properties of resins in oral fluid exposure [21,22]. The main outcome of this study was a significant reduction of the VAS values after the application of the agents compared to the baseline values. The sensitivity relief by VF significantly decrease immediately after the application, remaining almost unchanged during the 4 week controls ranging in 70% of efficacy compared to the baseline values. After 8 weeks, it was observed an increase of sensitivity and VAS values compared to the 4 weeks values. Result may be related to a progressive decrease of VF seal under environment. The significant decrease of the VAS values in POST 1 may be related to the chemical and physical bonding of VF in dentine due to its acidic monomer composition and the presence of chemical elements able to enhance the adhesion capacity of the self adhering composite [14]. The acidic phosphate group in the monomer composition of VF may have raised the concentration of Ca and P from the dentine allowing a precipitation of Ca and P on the dentine with micromechanical interactions on the surface. It is like that the same chemical components of VF matrix have caused the progressive decrease of the seal in dentin raising the sensitivity at the 12 week control.

It has been stated that resin based materials absorb water in an aqueous environment, leading to deterioration of the physico-mechanical properties, mainly due to a hydrolytic breakdown of the bond between silane and filler particles, filler-matrix debonding or even hydrolytic degradation of the fillers [23] Hydrolytic breakdown was mostly related to the presence of Si, Ba, Al [24] which are usually added to increase radio-opacity, shorten the setting and increase hardness in resins.

Si, Yt, F, and Ba leachable ions high represented in VF matrix, may have allowed permeation of water molecules into the resin spaces previously occupied by those ions [23] leading to a reduction of the strength of resin–filler interface which may have weakened the mechanical properties, and the chemical bond of VF in dentine [25], justifying the progressive decrease of the sealing at 12 weeks control of the VAS.

UDS revealed Ca, Cl, and Si as the highest ions in the resin matrix also containing Al ion peaks. Clinically, the behavior of the resin sealant was different to that of the self-adhesive composite. UDS produced a slow decrease of the VAS values in the weeks compared to the baseline, raising to about 87% of the pain reduction at the 12 week controls in comparison to the 73% of reduction obtained after the application of VF. Results may be related to different leachable ions composition and filler treatment in UDS which may indicate that the filler-polymer bond was less attacked by hydrolytic degradation under oral exposure. Moreover, the different behavior of UDS in respect to VF, may indicate that the 12 weeks of oral environment conditions would be essential for the UDS sealing expression itself.

In the case of CPB, the presence of Si, P, Al and F ions can be explained in the acidic methacrylate composition of the self etching enriched by filler particles of fluorine-containing reactive silicate glasses [26]. Clinically, CPB showed a significant decrease of the VAS values in Post 1 with a reduction of efficacy within the 12 week controls. CPB efficiency is moreover related to the chemical performance of the functional monomer MDB contained in the primer which is able of ionic interactions with calcium in dentine [27] and glass-ionomer acid–base reactions with the filler of fluoride-allumin silicate glass contained in the adhesive [26]. Nevertheless, the reduction of efficiency in CPB seal in the time of oral exposure may be explained in the incapacity of this bonding in dentine to resist in face to the oral stress unless a composite cover is performed [28].

FOV reduction of VAS values is explained by the presence of a cover of varnish on dentine with he precipitation of crystallites of calcium fluoride or phosphate containing calcium fluoride in the opening of the tubules [4,12]. This mechanism of working, observed under SEM [14] was able to reduce the tubular apertures in exposed surface allowing for a decrease of tubular fluid conductance in POST 1. However, the progressive decrease of efficacy is related to the inability of a firm seal in dentine. Moreover the decrease of efficacy may be correlated to the release of the F ions in environment compromising the integrity of the material in water [12,29].

As a result of this investigation, all the materials tested produced a reduction of dentine permeability. However, after 12 week controls, there was no statistically significant difference in the decrease of the VAS irrespective of the desensitising agent employed. Thus, the null-hypothesis was accepted.

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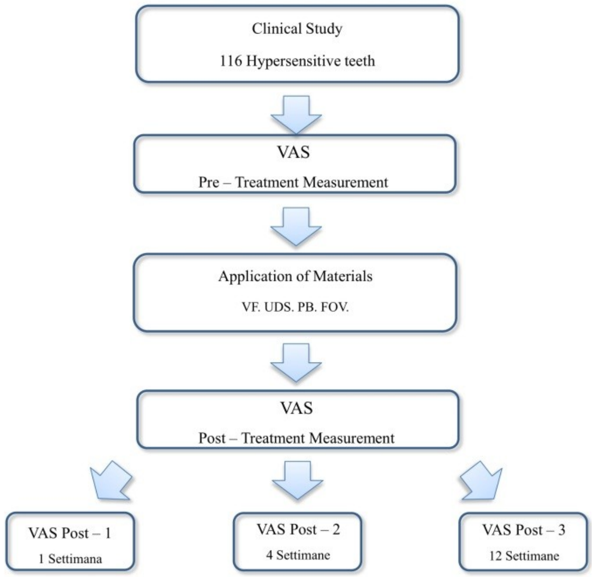

Fig 1 – Summary of the experimental design to collect hypersensitivity teeth and test different desensitising materials for the SEM morphological study and the clinical study.

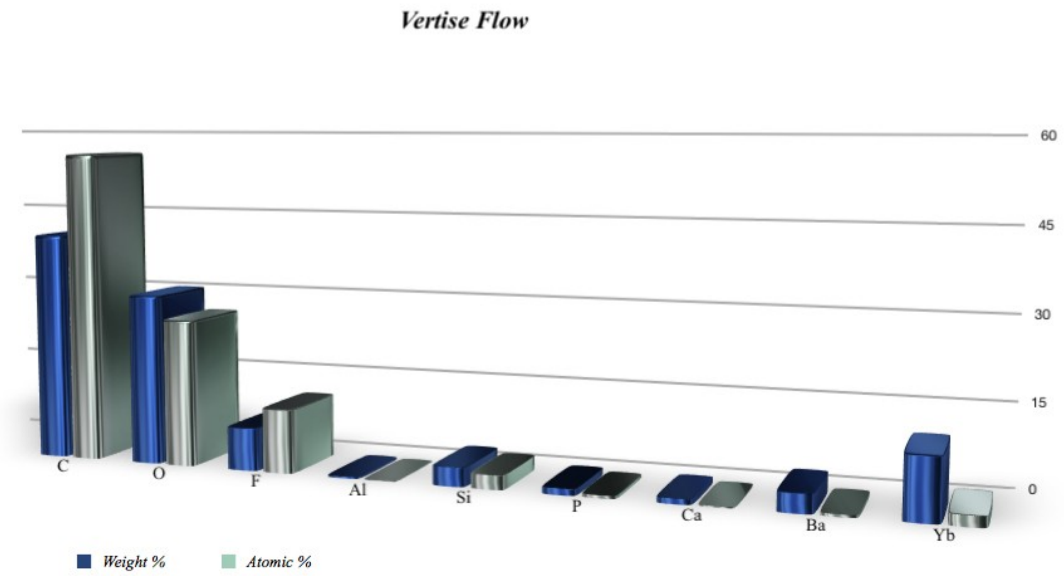

Fig 2 – The mean values and standard deviations of the atomic weight percentage of ions present in VF.

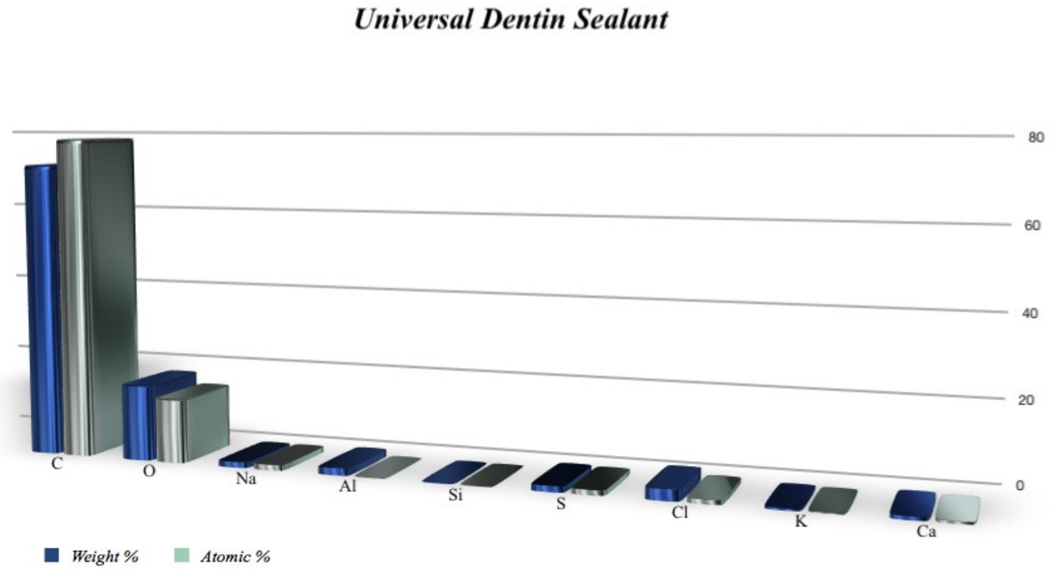

Fig 3 – The mean values and standard deviations of the atomic weight percentage of ions present in UDS

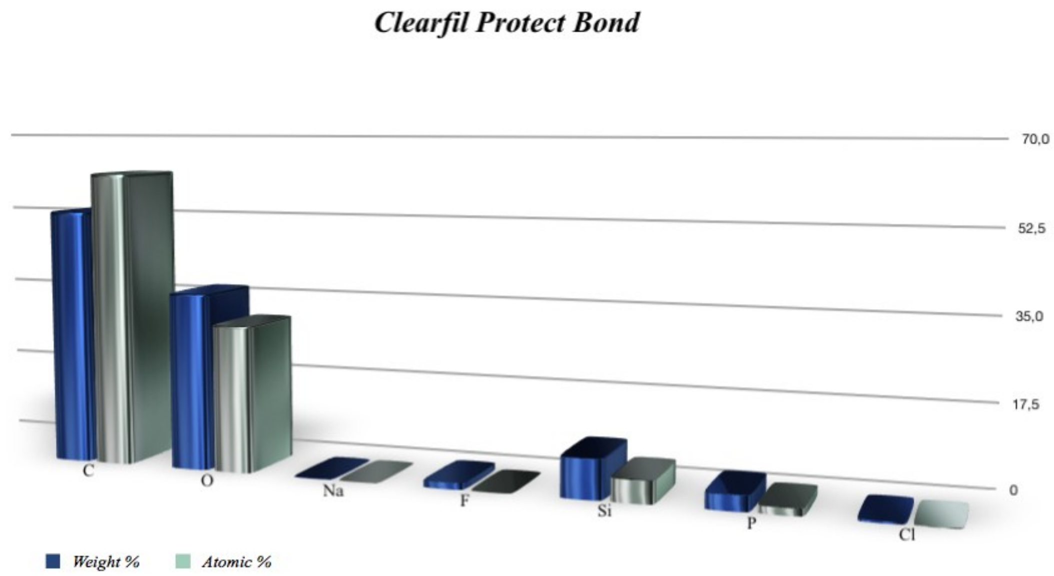

Fig 4 – The mean values and standard deviations of the atomic weight percentage of ions present in CPB.

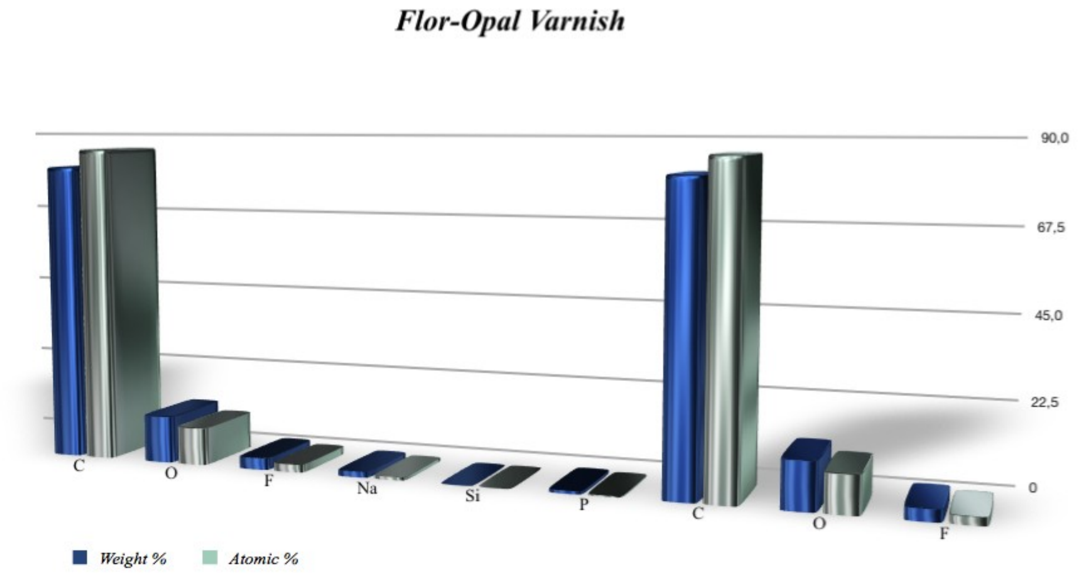

Fig 5 – The mean values and standard deviations of the atomic weight percentage of ions present in FOV.

Table 1. Visual Analogue Scale (VAS) values measured in 30 patients baseline and post-treatment. Values expressed as means and standard deviation.

VAS	VF Mean ±SD	UDS Mean ±SD	PB Mean ±SD	FOV Mean ±SD	One way ANOVA p-value
t0 (before application)	3.75 (± 1.57)	4.66 (± 1.88)	4.60 (± 1.97)	4.00 (± 1.73)	0.21
t1 (after application)	0.85 (± 0.93)	2,85 (± 1.79)	2.13 (± 1.54)	2.35 (± 1.17)	0.02
t2 (7 days after application)	1.07 (± 0.90)	2.25 (± 1.31)	2.16 (± 1.72)	2.00 (± 1.00)	0.01
t3 (30 days after application)	2.03 (± 1.77)	2.40 (± 1.50)	2.40 (± 1.35)	2.51 (± 0.96)	0.18
t4 (90 days after application)	2.57 (± 1.13)	2.18 (± 1.17)	2.66 (± 1.12)	2.48 (± 0.99)	0.22
ANOVA within groups p-value	0.01	<0.01	0.03	<0.01	

* VAS = Visual Analogue Scale of Pain

VF= Vertise Flow

UDS= Universal Dentine Sealant

PB= Protect Bond

FOV= Flor-Opal Varnish