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Research

Comparison of Two Tricalcium Phosphate Varnishes and a Comparator Fluoride Varnish on Tubular Occlusion

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Abstract

Objectives

The aim of the present study involved comparing the tubular occluding properties of three varnishes, two Clinpro™ varnishes and a Colgate® Duraphat® Varnish.

Method

Nine caries-free premolar dentine discs were prepared and treated with the three varnishes and subsequently observed under SEM at 0º–and 90º angle to assess their ability to cover the dentine surface. The tubule occluding properties were measured using a modified Pashley cell hydraulic conductance model. A further 9 caries-free extracted molars were sectioned into 500µ thick dentine discs. The fluid flow rate was assessed after a) immersing the discs in 6% citric acid for 2 minutes, b) treating the dentine disc with the experimental varnishes and c) subjecting the treated discs to an acid challenge (6% citric acid for 2 minutes).

Results

SEM investigation showed uniform occlusion of the dentinal tubules, with varying depths of penetration. Hydraulic conductance tests showed no statistically significant differences in the fluid flow rate (expressed as percentages) when all the three varnishes were compared at different stages of treatment ($p = 0.33$). However, after subjecting the discs to an acid challenge, there was a statistically significant increase in the fluid flow rate with the Colgate® Duraphat® Varnish treated discs, whereas the Clinpro™ White Varnish and Clinpro™ XT Varnish discs showed no statistically significant differences ($p = 0.99$ and $p= 0.83$ respectively).

Conclusions

All the tested varnishes (Colgate® Duraphat® Varnish, Clinpro™ White Varnish and Clinpro™ XT varnish) were effective in blocking the dentinal tubules as demonstrated in this in vitro study. However, the tricalcium phosphate varnishes (Clinpro™) were more resistant to an in vitro acid challenge compared to the sodium fluoride varnish (Colgate® Duraphat®).

Key Words: Tricalcium Phosphate Varnishes Fluoride Varnish; Tubular Occlusion; Hydraulic Conductance; Dentine Hypersensitivity

Introduction

Dentine Hypersensitivity (DH) is a clinical problem that can impact on the quality of life of individuals who may experience discomfort when having hot and cold food and drinks during their day to day activities. The reported prevalence rates in the published literature range from 3.8%-74%, although this figure is dependent to some extent on the country in which individuals were examined and the methodology used to collect the data [1]. Although there are a plethora of products and treatment procedures available to treat DH, it is apparent that there is no ideal desensitizing product either over-the-counter [OTC] or professionally applied [In-office] that provides both fast acting and long lasting protection against the pain associated with DH. The most accepted theory for reducing DH is the hydrodynamic theory [2] and the action of most of the toothpaste formulations, gels, varnishes, mouthwashes and restorative materials are based on either their 1) tubular occluding or 2) nerve desensitization properties. Several investigators [3, 4] have previously attempted to provide some recommendations for an ideal desensitizing product from a clinical perspective. However, it is apparent from the

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published literature that there does not appear to be an ideal desensitizing product suitable for every clinical actuality [5]. Historically a plethora of products have been recommended for the treatment of DH, for example fluoride, calcium phosphate compounds, strontium and potassium containing-products, amorphous calcium phosphate [ACP] and tooth mousse ([casein phosphopeptide-amorphous calcium phosphate CPP-ACP]), have been commercially available during the last 40-50 years [6,7]. Historically Fluoride varnishes have been professionally applied in both the deciduous (milk) and permanent dentition for the prevention of dental caries [8]. More recently, products containing functionalised tricalcium phosphate (fTCP) have been made available for the treatment of several dental conditions such as remineralisation of enamel, reducing dentine sensitivity, prevention of demineralisation around orthodontic brackets as well as in placing resin sealants in occlusal fissures for paediatric dentistry [9,10]. Several in vitro studies have demonstrated that Tricalcium phosphate products occlude the dentinal tubules which, if reproduced in the clinical environment would reduce/or relieve dentine hypersensitivity [9, 11].

Aim of the Study

The aim of this in vitro study was to compare the tubular occluding properties of three varnishes, namely Clinpro™ varnish with fluoride (Clinpro™ White Varnish [3M ESPE], a resin modified glass ionomer based varnish, Clinpro™ XT [3M ESPE]) and a comparator fluoride varnish, Duraphat® (Colgate-Palmolive, Ltd, U.K). The three products were assessed using Scanning Electron Microscopy (SEM) on their ability to cover the dentine surface and occlude the dentine tubules. (SEM) and fluid flow measurements (hydraulic conductance) in a modified Pashley cell were also used to assess the three varnishes. The durability of the three commercial products following an acid challenge was also assessed.

Materials and Methods

Materials

Three commercially available varnishes namely, Clinpro™ White Varnish (3M ESPE) based on fTCP technology, Clinpro™ XT Varnish (3M ESPE), a resin modified glass ionomer based varnish with fluoride and a sodium fluoride varnish, Duraphat® (Colgate-Palmolive, Ltd, U.K) were assessed for tubular occluding properties and reduction in fluid flow through dentine. The varnishes were applied to the dentine surface following the Manufacturers' instructions for clinical use.

Method

Nine extracted caries free maxillary and mandibular premolars and nine caries free maxillary and mandibular molars were obtained from the tooth bank at the Royal London Dental Hospital, Whitechapel, London (Ethics approval was granted for the use of human tissue at QMUL).The teeth were stored in 70% ethyl alcohol (w/v) until they were required for the study. The premolar teeth were sectioned mesio-distally into discs approximately 1 mm thick using a diamond blade (Microslice annular blade, Ultratec, USA) on a cutting machine (Struers Accutom-5 diamond cutting machine). This section was obtained from the middle third of the crown (mid coronal).The dentine discs were placed in an ultrasonic bath for 30 seconds to remove any residual smear layer developed from the polishing process. They were treated with 6% citric acid for 2 minutes (w/v) to remove any remaining smear layer and then rinsed with distilled water before usage. The premolar dentine discs were fractured using orthodontic pliers to produce two equal halves, one half would be nominated as a 'control', the other as the 'test' half. Both the test and control halves of the discs (premolar teeth) were dried in a fume cupboard overnight to allow for desiccation of the discs prior to the application of the test products on the surface of the dentine disc in preparation for SEM evaluation.

The molar dentine discs were cut to a 600µ thickness and polished with P180, P400 and P4000 paper to a thickness of 450 - 550µ, measured using a digital Vernier caliper (Manufacturer: Sealey, Model NO: AK962EV). The dentine discs (Molar teeth) were placed in an ultrasonic bath for 30 seconds to remove any residual smear layer developed from the polishing process. These discs were treated with 6% citric acid for 2 minutes (w/v) to remove any remaining smear layer and then rinsed with distilled water prior to using a modified Pashley Hydraulic conductance model. This model measures the fluid flow rate (hydraulic conductance [Lp]) through the dentine discs to evaluate the effectiveness of the test products in reducing fluid flow through the tubules.

Evaluation of Dentine Specimens by Tubule Occlusion (Scanning Electron Microscopy [SEM])

The methodology described by Gillam et al. [12] was followed. The premolar discs were splutter coated with gold dust and then aligned together to replicate one whole disc. These discs were viewed under SEM (Field Emission Inspection, Oxford instruments, Oxfordshire, UK) using a secondary scanning imaging voltage of 10 KV, at a distance of 10 mm.

Effectiveness of Tubule Occlusion Using Hydraulic Conductance (Fluid Flow) Measurements

A modified Pashley cell was used to determine the fluid flow through each of the dentine discs [13]. The discs from the molar specimens were used to assess the fluid flow through the individual disc following etching for 2 minutes with 6% citric acid. This was repeated after applying the different varnishes on the discs and subsequently subjecting them to an acid challenge (6% citric acid for 2 minutes).

Results

Scanning Electron Microscopy (SEM) of The Effects of The Test And Control Products On Tubule Occlusion

Figures 1-3 show SEM images of both the control and test specimens of the dentine discs at different magnifications. The open dentinal tubules can be observed on the control specimen, after the discs were treated with 6% citric acid. Complete occlusion of the surface of the dentinal tubules can also be observed following treatment with all the three varnishes. Typical crystal particles of the resin cement from the Clinpro™ XT Varnish can be observed penetrating the dentinal tubules at x10,000 magnification. Colgate® Duraphat® varnish can also be observed in the dentinal tubules as seen in this vertical cross-section.

Hydraulic Conductance Fluid Flow Measurements.

The fluid flow reduction (FFR) results for the dental varnishes were recorded in Tables 1-3. One way ANOVA tests ($P < 0.05$) were used to analyse the data from the results.

The mean fluid flow rate of each varnish at difference stages of treatment was assessed. One-way ANOVA and post hoc Tukey's tests were performed to evaluate the treatment comparisons between a single varnish.

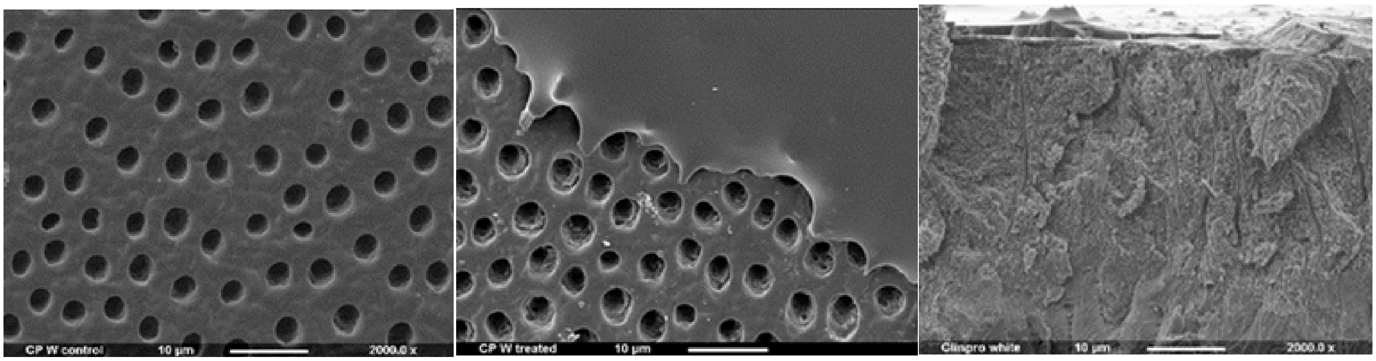

Fig. 1- SEM image of a) control dentine section, b) section treated with a Clinpro™ White Varnish c) a fractured section treated with a Clinpro™ White Varnish [x2000 Magnification]

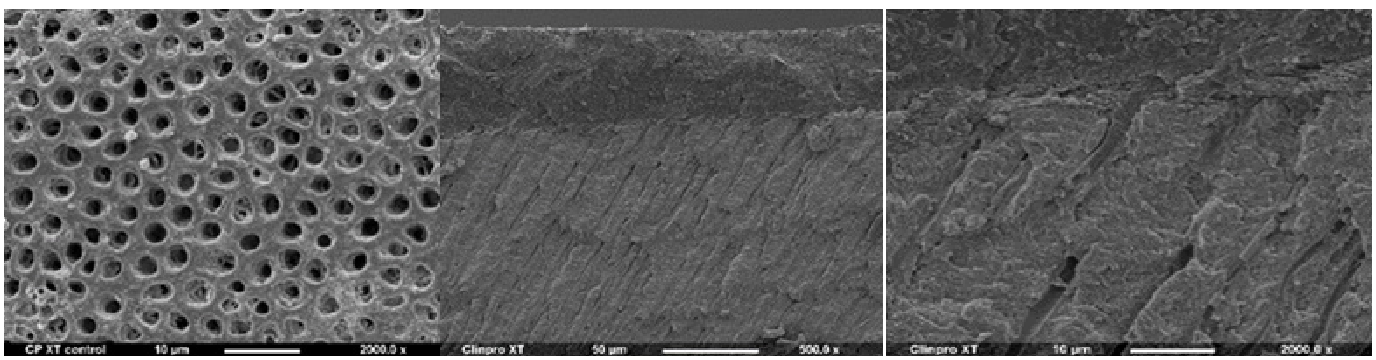

Fig.2a-c SEM image of a) Dentine disc treated with Clinpro™ XT Varnish [x2000 magnification] b) Dentine disc treated with Clinpro™ XT Varnish [x10,000 magnification] c) Cross section of a disc treated with Clinpro™ XT varnish [10,000x magnification]. Note the crystal-like inclusions which penetrate into the dentinal tubules

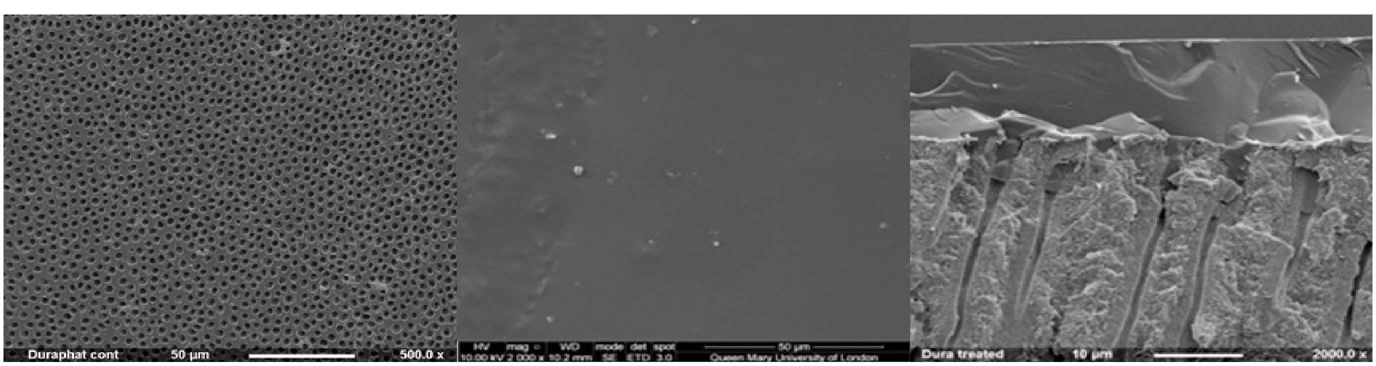

Figure 3a-c SEM image of a) Control dentine section , b) surface coverage following application of the Colgate® Duraphat® varnish and c) a test disc fractured cross-section treated with Colgate® Duraphat® Varnish [2000x magnification]. (Note the penetration of the material into the dentinal tubules).

Table 1 - Comparison of the mean of the fluid flow rates of dentine discs treated with Clinpro™ White Varnish - a) Baseline 6% citric acid challenge b) after applying the varnish c) after subjecting the dentine disc to an acid challenge

Treatment (I)	Treatment (J)	Mean Difference (I – J)	Std. Error	Sig.	95 % confidence Interval	
					Lower Bound	Upper Bound
Baseline 6% Citric Acid challenge	After applying varnish	10.47667*	2.04762	0.009	3.8139	17.1394
	Subjecting to acid challenge	10.22000*	2.28931	0.150	2.7708	17.6692
After Applying varnish	6% citric acid	-10.47667*	2.04762	0.009	-17.1394	-3.8139
	subjecting to acid challenge	-0.25667	2.28931	0.993	-7.7059	7.1925
Subjecting to an acid challenge	6% citric acid	-10.22000*	2.28931	0.015	-17.6692	-2.7708
	After applying varnish	0.25667	2.28931	0.993	-7.1925	7.7059

* - The mean difference is significant at 0.05 level

Table 2: Comparison of the mean of the fluid flow rates of dentine discs treated with Clinpro™ XT - a) Baseline 6% citric acid challenge b) after applying the varnish c) after subjecting the dentine disc to an acid challenge

Treatment (I)	Treatment (J)	Mean Difference (I – J)	Std. Error	Sig.	95 % confidence Interval	
					Lower Bound	Upper Bound
Baseline 6% Citric Acid challenge	After applying varnish	8.98000	1.27342	0.001	5.0728	12.8872
	Subjecting to acid challenge	8.23000	1.27342	0.002	4.3228	12.1372
After Applying varnish	6% citric acid	-8.98000	1.27342	0.001	-12.8872	-5.0728
	subjecting to acid challenge	0.75000	1.27342	0.831	-4.6572	3.1572
Subjecting to an acid challenge	6% citric acid	-8.23000	1.27342	0.002	-12.1372	-4.3228
	After applying varnish	0.75000	1.27342	0.831	-3.1572	4.6572
* - The mean difference is significant at 0.05 level						

Table 3 - Comparison of the mean of the fluid flow rates of dentine discs treated with Colgate® Duraphat® Varnish - a) Baseline 6% citric acid challenge b) after applying the varnish c) after subjecting the dentine disc to an acid challenge.

Treatment (I)	**Treatment (J)**	**Mean Difference (I – J)**	**Std. Error**	**Sig.**	**95 % confidence Interval**	
					Lower Bound	**Upper Bound**
Baseline 6% Citric Acid challenge	After applying varnish	7.34000*	0.34829	0.000	6.2714	8.4086
	Subjecting to acid challenge	6.29667*	0.34829	0.000	5.2280	7.3653
After Applying varnish	6% citric acid	-7.34000*	0.34829	0.000	-8.4086	-6.2714
	subjecting to acid challenge	-1.04333	0.34829	0.055	2.1120	0.0253
Subjecting to an acid challenge	6% citric acid	-6.29667*	0.34829	0.000	-7.3653	-5.2280
	After applying varnish	1.04333	0.34829	0.055	-0.0253	2.1120

* - The mean difference is significant at 0.05 level

Discussion

It is evident from the published literature that despite the numerous products and techniques used to treat DH there still appears to be no ideal product that is universally accepted [5,7] Previously Grossman [4], recommended that the ideal desensitizing agent should not irritate or endanger the integrity of the pulp. It should be relatively painless on application or shortly afterwards, should be easily applied, be rapid in action and finally should not discolour the tooth structure. Hill & Gillam [5] suggested that both Grossman [4] and Gillam [6] attempted to provide recommendations for an ideal desensitizing product from a clinical rather than a chemico-physical perspective.

Desensitising agents work on a range of mechanisms such as nerve desensitisation, protein precipitation, plugging dentinal tubules, dentine adhesive sealers to lasers and homeopathic medication [8]. Fluoride products have been shown to occlude dentinal tubules using in-vitro studies [9]. The findings determined from both SEM and hydraulic conductance studies, assessed the effectiveness of the Clinpro™ White Varnish, Clinpro™ XT varnish and Duraphat® varnish (Figs 1-3, Tables 1-3). The dentine disc model may accurately be used in in vitro experiments to assess dentine hypersensitivity in relation to the application of various products on to a dentine surface [10-11]. Sensitive dentine has been recognised to have wider dentinal tubules than non-sensitive dentine as reported in the Absi et al. Study[14], therefore it is reasonable to expect that occluding dentinal tubules by a desensitising agent with tubular occluding properties is likely to reduce sensitivity (as proposed in the Hydrodynamic theory) in a clinical setting. The use of acid etching in the in vitro model therefore, is an attempt to mimic the clinical situation. The initial acid etching was performed using 6% citric acid to remove the smear layer and open the dentinal tubules but it could also be argued that it may degrade protein and other constituents of dentine that would be required for chemical bonding etc., [13]. The choice of using premolars for the SEM and molars for the hydraulic conductance measurements was due, in part to the lack of availability of suitable molar teeth whereas there was an abundance of premolars extracted for orthodontic purposes. The dimensions of the premolar however, were too small for the Pashley cell, hence the molar teeth were reserved solely for the hydraulic conductance measurements.

From the SEM evaluation (Figure. 1) of the Clinpro™ White Varnish, a uniform surface occlusion of the dentinal tubules was observed which was similar to the images obtained from the Clinpro™ White Varnish (3M ESPE) brochure. An in vitro SEM study by Tosun et al. also reported that an application of Clinpro® White Varnish occluded the dentinal tubules, although there was an increased effect on tubular occlusion when using a combination of Clinpro™ White Varnish with a Nd:Yag laser [15].

In the present investigation the Clinpro™ XT product however, completely covered the surface of the dentinal tubules with resin tags penetrating into the dentinal tubules (Figure. 2)

Furthermore, the Duraphat® varnish demonstrated almost complete occlusion of dentinal tubules which would suggest that the varnish would be effective as a tubular occluding agent (Fig. 3). This observation was consistent with other investigators who reported that sodium fluoride varnishes uniformly occluded majority of the tubules [16,17]. Penetration of all three varnishes into the dentine tubules was also evident from the fractured cross sections (Figures. 1-3). The permeability of the dentinal tubules following tubule occlusion can also be determined by the ease in which the fluid flows within the dentinal tubules. This can be recorded using the Pashley cell, where both the tubular fluid movements can be measured before and after occluding the dentine disc with a test product [13,18]. The rationale behind using both SEM and hydraulic conductance when testing for the effectiveness of a desensitizing product was to evaluate the full extent of a product's ability to completely block the dentinal tubules. This can be then related to its ability to block the fluid flow through the dentine disc.

The results of the present study would suggest that all three dentine varnishes were effective in occluding the dentinal tubules in an in vitro environment. Furthermore, the SEM images also demonstrated that the products penetrated into the dentinal tubules which would suggest that the varnishes not only covered the dentine surface but also occluded the dentinal tubules.

There was a marked reduction in the fluid flow rate through the dentinal tubules (hydraulic conductance model) following the application of all three varnishes. A maximum decrease in the original fluid flow rates (e.g., before the application of the varnishes) were observed with Clinpro™ White Varnish, followed by Clinpro™ XT and lastly by the Duraphat® varnish. Therefore, this data support the observation in the present investigation that all three varnishes had occluded the dentinal tubules.

After the acid challenge, the only varnish with a statistically significant increase in the fluid flow rate (compared to the fluid flow rates prior to the acid challenge) was the Colgate® Duraphat® Varnish (Table 3). This observation would suggest that these two ClinPro™ varnishes were more resistant to an in vitro acid challenge compared to the Duraphat® varnish.

Conclusions

All three dentine varnishes were effective in occluding the dentinal tubules in an in vitro environment, as observed by SEM. Images from the cross sectional (fractured) sections also showed that the products penetrated below the surface of the dentine into the dentinal tubules. This would suggest that the varnishes not only covered the dentine surface but also occlude the dentinal tubules. The results from the hydraulic conductance measurements would suggest that the two ClinPro™ varnishes were more resistant to an in vitro acid challenge compared to the Duraphat® varnish.

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